Myogenic and Neurogenic Factors in the Control of Pyeloureteral Motility and Ureteral Peristalsis

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I. Introduction

The main, and possibly only, function of the pyeloureteral complex is to ensure the unidirectional transport of urine from the kidney to the urinary bladder. An extremely efficient set of mechanisms operates to achieve this target: the basic process regulating ureteral peristalsis is myogenic, being initiated by spontaneously active pacemaker cells in the renal pelvis. In recent years, the mechanisms of excitability and electromechanical coupling in ureter cells have been extensively investigated; several pharmacological tools have been instrumental for a further understanding of the myogenic regulation of pyeloureteral motility.

A role of innervation in regulating pyeloureteral motility has been advocated from time to time (See Boyarsky and Labay, 1969; Weiss, 1992; Amann, 1993) but its definition has remained elusive until recently. In particular, a convincing proof for a local control of ureteral motility by cholinergic or noradrenergic nerves has not been obtained. The existence of a prominent sensory innervation of the renal pelvis and ureter is self-evident when considering that renal colic arising from the presence/passage of a stone along the ureter is one of the most intense and vivid forms of visceral pain. In the past few years, important advancements have been made through the recognition of the role of sensory innervation in determining local changes of pyeloureteral motility and in producing neurogenic inflammation.

In both areas of investigation (myogenic and neurogenic regulation of pyeloureteral function), the use of novel drugs and pharmacological tools has been instrumental to unravel principles of physiological or pathophysiological relevance. Our purpose is to bring together the new information obtained regarding the myogenic and neurogenic factors regulating pyeloureteral motility, ureteral pain, and inflammation and to discuss this recently obtained information on the background of the previous literature.

II. The Myogenic Theory of Ureteral Peristalsis

The roots of the myogenic theory of ureteral peristalsis can be traced back to the pioneering work of Engelmann (1869) who localized the origin of peristaltic pressure waves in the renal pelvis and proposed that the impulse for ureteral contraction passes from one ureter cell to another, the whole ureter working as a syncitium. The basic organization of the pyeloureteral complex has a loose resemblance to that regulating cardiac excitation and contractility (fig. 1). In both organs, the electrical and mechanical activities are initiated by spontaneously active cells (in the atrium and renal pelvis, respectively) and are then conducted to regions that are not normally active unless driven by a pacemaker (the ventricles and ureter, respectively); in addition, both the ureter and cardiac tissues have a plateau-type action potential (Bozler, 1942a,b; Burnstock and Prosser, 1960). However, as evidenced at several places in this review, the analogy holds true only at a very superficial level.

The main pieces of evidence indicating that ureteral peristalsis is essentially a myogenic process are as follows: (*a*) peristalsis occurs in the isolated pyeloureteral complex with characteristics similar as those observed in vivo, providing that the renal pelvis is attached to the ureter (Macht, 1916; Finberg and Peart, 1970; Malin *et al.*, 1970); (*b*) an efficient peristalsis occurs, with characteristics similar to those observed before surgery, after denervation of the ureter (Wharton, 1932), in autotransplanted ureters (O'Conor and Dawson-Edwards, 1959), and even in a reversed ureter (Melick *et al.*, 1961); (*c*) peristalsis of the isolated pyeloureteral tract is unaffected by application of tetrodotoxin or other neuron blocking agents (Golenhofen and Hannappel, 1973; Teele and Lang, 1998).

A. The Pacemaker Function of the Renal Pelvis

Some debate has developed in the literature about the exact localization of a putatively unique pacemaker responsible for initiation of peristaltic waves. Most authors have localized *the* pacemaker in supraureteral structures (calyces and proximal renal pelvis) (Benjamin, 1971; Constantinou, 1978; Kiil, 1957; Hannappel and Golenhofen, 1974a; Morita *et al.*, 1981; Tsuchida *et al.*, 1981). Kobayashi (1964, 1965) found the pacemaker at the border between the renal pelvis and calyces. Golenhofen and Hannappel (1973) presented evidence indicating that the utmost renal ends of the renal pelvis are involved in the pacemaker process. The other prevailing opinion indicated the pyeloureteral junction as the main pacemaker (Barr, 1971; Weiss *et al.*, 1967; Weiss, 1971). The issue about the location of the pacemaker is complicated by the existence of remarkable species-related differences in the anatomical organization of the renal pelvis and calyces (Kiil and Setekleiv, 1973).

Because a frequency gradient exists in the renal pelvis in terms of autorhythmicity, the most proximal portions
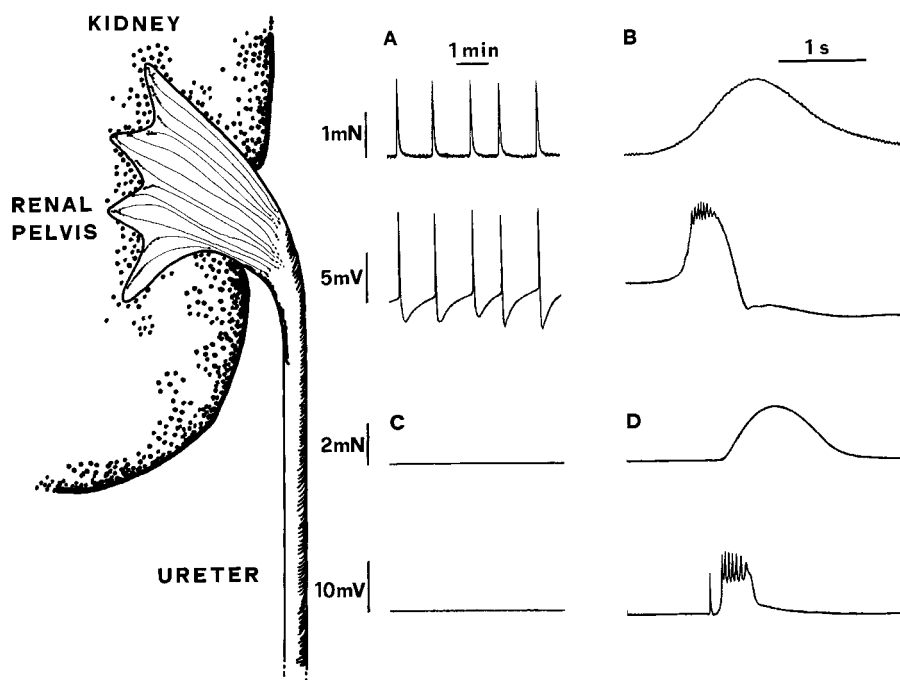
FIG. 1. Schematic drawing illustrating the basic organization of the pyeloureteral complex (renal pelvis/ureter). Ureteral peristalsis originates from the renal pelvis where urine produced in the kidney collected: the renal pelvis is spontaneously active (panels A and B) to generate a pacemaker activity which propagates to the ureter thus determining the propulsion of boluses of urine toward the bladder. The ureter is normally quiescent (panel C) although latent pacemakers exist at all levels of the pyeloureteral tract which can be excited to fire action potentials (panel D) and contractions by depolarizing stimuli. Excitation of latent pacemakers by noxious/irritant stimuli can produce antiperistaltic waves of contraction producing urine reflux toward the kidney.

of the renal pelvis are thought to generate the physiological rhythm of peristalsis. The existence of specialized cells capable of generating a spontaneous rhythm in the renal pelvis had been suggested in early anatomical studies. Gosling and Dixon (1972, 1974) described special smooth muscle cells with pale-staining cytoplasm present in the renal calyx and pelvis but not in the ureter and discussed the possibility that these cells may have a special "pacemaker" quality. Lang *et al.* (1998) recently described the presence of fibroblasts-like cells, resembling the interstitial cells of Cajal that are responsible for pacemaking activity in the smooth muscle of the intestine, to be present in the proximal part of the guinea pig renal pelvis. Electrophysiological evidence also indicates an enrichment in the density of pacemaker cells at the level of the proximal renal pelvis, as reviewed in Section III.B.

According to the current data, it seems that the whole renal pelvis acts as a pacemaker to generate all-or-none propulsion of urine and that a proximal-to-distal gradient exists for setting the frequency of spontaneous activity. When the proximal, middle, or distal renal pelvis was dissected, spontaneous activity was detected from each subregion with similar frequencies as those observed before dissection (Constantinou *et al.*, 1978; Constantinou and Yamaguchi, 1981), i.e., every subregion of the renal pelvis behaves as an oscillator.

A "multiple coupled oscillators" model has been developed to explain the ability of the whole renal pelvis to act

as the pacemaker and to respond to an increase in diuresis with an increased frequency of urine transport. According to this model, several pacemaker subunits exist in the renal pelvis, the oscillation of each subunit being insufficient to trigger peristalsis; coupling processes (mainly electrical) between the subunits facilitate the synchronization and summation of activity to increase the chance of triggering a conducted peristaltic wave (Golenhofen and Hannappel, 1973; Constantinou and Yamaguchi, 1981). According to this model, the force of the total pacemaker will depend on (*a*) the force of each subunit and (*b*) the degree of synchronization between subunits. Physical stimuli, such as the distention, modulate the peristaltic frequency by changing the degree of synchronization between subunits, possibly through the generation of prostanoids (Section III.E). This model can be applied independently from the species-dependent anatomical complexities of the calyces and renal pelvis.

Lammers *et al.* (1996) have analyzed the spatial and temporal variations in the pacemaker process by performing simultaneous extracellular electrophysiological recordings from a large number of sites in the whole isolated sheep renal pelvis. By reconstructing the initiation and spread of activity, they showed that the pacemaker was located at the pyelocalyceal border and never in the body of the pelvis or in the area of the pelvi-ureteric junction. One single pacemaker was invariably responsible for a particular spread of activation and

fusion of activity from two or more pacemakers did not take place. Spontaneous shifts of the pacemaker could occur from one site to another along the pyelocalyceal border. To what extent the observations of Lammers *et al.* (1996) in the sheep renal pelvis can be applied to other species is not known; the results contradict the hypothesis (Gosling and Dixon, 1974) that in species with multicalyceal kidneys, several pacemakers from distinct calyces could discharge simultaneously and their impulses could merge together into a single wavefront. Lammers *et al.* (1996) proposed that, in the intact renal pelvis, factors such as the degree of filling of a particular calyx or local stretch may determine the initiation of the pacemaker activity at a given site.

B. Propagation of the Pacemaker Signal

Excitation waves spread from the renal pelvis to the ureter, determining the propulsion of urine through an intermittent phasic-type contractility. Considering the ureter as a syncitium (Bozler, 1942a,b), the propagation of impulses occurs as a purely myogenic process (via electrotonic spread) at points of intimate contact between ureter muscle cells or "gap junctions" (Notley, 1968; Uehara and Burnstock, 1970; Tahara, 1990). This model predicts that the suppression of action potentials at any site of the ureter will suppress the propagation of contraction and peristalsis.

Lammers *et al.* (1996) analyzed the conduction of impulses in the sheep isolated renal pelvis. They observed that conduction from the site of initiation of the pacemaker current to the pyeloureteral junction is slow, inhomogeneous and contorted: multiple instances of partial or total conduction block were observed in the renal pelvis. In several cases, conduction block was related to the refractory period but in other instances no apparent relationship was evident between the occurrence of a conduction block and the length of preceding intervals. Lammers *et al.* (1996) speculated that a poor coupling between cells or stretch could be involved in conduction block within the renal pelvis.

A conduction block also exists between the renal pelvis and the ureter (Constantinou, 1974, 1978; Hrynczuk and Schwartz, 1975; Zawalinski *et al.*, 1975; Constantinou and Hrynczuk, 1976); under the conditions of a normal diuresis, every pacemaker contraction of the renal pelvis does not always propagate to the ureter, suggesting that a urine flow-dependent mechanism triggers ureteral peristalsis at the pelviureteric junction. Stretching forces exerted on the pyeloureteral region by accumulating urine increase the coupling strength until they enable an incoming "pacemaker" wave of excitation to pass to the ureter (Constantinou and Yamaguchi, 1981). With increasing urine flow rates, the frequency of peristaltic contractions reaches that of the pacemaker; at this stage, further increases in urine production are accommodated by bolus volume increases, until the ure-

ter assumes the form of an open duct (Constantinou *et al.*, 1974).

Regional variations in the excitability and in the rate of propagation of the peristaltic wave have been described in the guinea pig, cat, and rat ureter (e.g., see Kobayashi, 1965; Tindall, 1972; Tsuchiya and Takei, 1990; Weiss, 1992). By using a three-chamber partitioned organ bath (Meini *et al.*, 1995), we demonstrated that: (*a*) depolarizing stimuli applied at either end of the guinea pig ureter produce a propagated wave of excitation which travels to the other end of the organ; (*b*) the rate of propagation of contractions is independent of the site of application of the stimulus; (*c*) latent pacemakers capable of generating a propagated wave of excitation are present in all regions of the ureter; and (*d*) the amplitude of propagated responses is independent from the site and the nature of the depolarizing stimulus.

The findings of Meini *et al.* (1995) indicate also that, in principle, mechanical events are not essential for the propagation of impulses along the ureter. The main findings supporting this conclusion are as follows: (*a*) Bay K 8644 and glibenclamide, which produced a marked and a slight prolongation of action potential duration and enhancement of contraction, respectively (Maggi *et al.*, 1994a,b), had no effect on the propagation of impulses in the ureter; (*b*) 1 μM nifedipine produced a large (>80%) inhibition of the mechanical response at the site where depolarizing stimuli were applied without affecting the intensity of the propagated response: the suppression of the propagated response by nifedipine occurred in an all-or-none manner only when the response to directly applied stimuli was also suppressed; (*c*) drugs that suppress the action potential by producing hyperpolarization of the membrane [cromakalim, calcitonin gene-related peptide (CGRP)[b]] suppressed the propagated response: both the suppression of the propagated response and its recovery upon washout of cromakalim or CGRP occur in an all-or-none manner (Meini *et al.*, 1995).

[b] Abbreviations: 4-AP, 4-aminopyridine; AChE$^+$, acetylcholine sterase-positive; Ba, barium ions; CA, calcium ions; [Ca]$_i$, intracellular Ca concentration; cAMP, adenosine 3'5' cyclic-monophosphate; cGMP, guanosine 3'5' cyclic-monophosphate; CGRP, calcitonin gene-related peptide; CICR, calcium-induced calcium release; COX, cyclooxygenase; DBH, dopamine-b-hydroxylase; DHP, dihydropyridine; EFS, electrical field stimulation; H, hydrogen ions; IBMX, 3-isobutyl-1-methylxanthine; I$_{Ca}$, inward calcium current; I$_K$, voltage-dependent Ca-insensitive K current; I$_{K(Ca)}$, Ca-dependent K current; I$_{TO}$, transient outward K current; i.j.p., inhibitory junction potential; IMG, inferior mesenteric ganglion; K, potassium ions; [K]$_O$, extracellular K concentration; K$_{ATP}$, ATP-sensitive K channels; Li, lithium ions; Mn, manganese ions; Na, sodium ions; [Na]$_i$, intracellular Na concentration; [Na]$_o$, extracellular Na concentration; NO, nitric oxide; NKA, neurokinin A; NPY, neuropeptide Y; PKA; protein kinase A; pH$_i$, intracellular proton concentration; Rp-cAMPS, Rp-cAMP monophosphothioate; sEPSP, slow excitatory postsynaptic potential; SHR, spontaneously hypertensive rats; SP, substance P; STOCs, spontaneous transient outward currents; TEA, tetraethylammonium; TH, tyrosine hydroxylase; VIP, vasoactive intestinal polypeptide.

Altogether, these findings indicated that even marked "local" changes in action potential shape/duration and contractility do not affect or impair the propagation of impulses. In other words, action potentials that are profoundly altered in their characteristics are capable of sustaining the propagation of impulses through the functional syncitium of the ureter. This conclusion does not exclude the possibility that, in physiological conditions, the stretch of the ureteral wall produced by the advancing bolus of urine may affect or modulate excitability and peristalsis.

C. Latent Pacemakers and Antiperistalsis

Every single cell of the guinea pig ureter can fire an action potential in response to depolarization (Imaizumi et al., 1989a). Therefore, when applying stimuli of threshold strength, all ureter smooth muscle cells can, in principle, act as pacemakers. Action potentials produced at any site along the ureter will propagate ortho- and antidromically to determine propagated waves of excitation/contraction, i.e., peristalsis or antiperistalsis (Meini et al., 1995) (fig. 2).

The activation of latent pacemakers in the ureter by chemical agents may be an important event in pathophysiological conditions because antiperistaltic waves of excitation can be generated in this way (Weiss, 1992). Latent pacemakers in the ureteral smooth muscle can be excited to fire action potentials and generate propagated contractions by mediators released from intramural nerves (neurokinin A, noradrenaline) or mast cells (histamine, serotonin) (Shuba, 1977a,b; Maggi et al., 1986;

1988a; Dodel et al., 1996; Iselin et al., 1996; Patacchini et al., 1998) and by chemical mediators normally present in the urine (bradykinin, endothelin-1) or by bacterial peptides produced in the urine during infections (Maggi et al., 1992a).

III. Myogenic Factors Regulating Motility of Renal Pelvis and Ureter

A. Action Potentials Recorded from the Smooth Muscle of the Pyeloureteral Tract

1. Action potentials in the renal pelvis. Zawalinski et al. (1975) reported that action potentials recorded from the renal pelvis exhibit a markedly different waveform as compared with those recorded from the ureter smooth muscle. In particular, "spontaneous" or pacemaker action potentials are recorded from the renal pelvis (fig. 1): these display a simple waveform with a slowly developing depolarization or "prepotential" preceding a slow spike. Seki and Suzuki (1990) reported that slow waves recorded from the smooth muscle of the rabbit renal pelvis are resistant to cholinergic, noradrenergic, and neuronal blockers to suggest a purely myogenic origin. Reduction of the extracellular concentration of sodium ($[Na]_o$) or of calcium ions ($[Ca]_o$) inhibited the generation of slow waves.

Lang's group (Zhang and Lang, 1994; Lang et al., 1995; Lang and Zhang, 1996) recently reported single cell electrophysiological recordings from the guinea pig renal pelvis. They described three distinct types of cells on the basis of the different action potentials that were
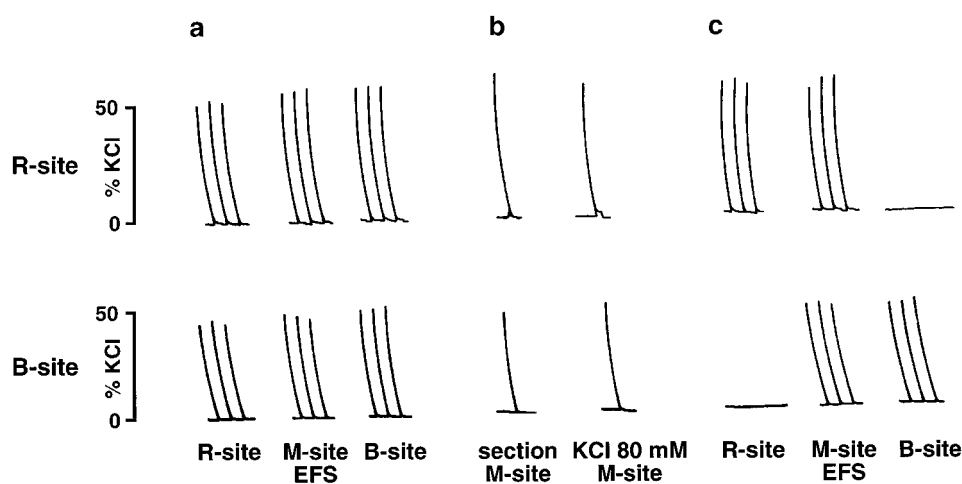
FIG. 2. Experiments illustrating the concept that latent pacemakers capable of generating a propagated wave of contraction are present at all sites of the ureter smooth muscle. The experiments were performed as described in Meini et al. (1995): the whole guinea-pig ureter was placed horizontally in a water-jacketed perspex bath for isolated organs. Two perspex partitions were used to separate the renal-, middle- and bladder-sites (R-, M- and B-site, respectively). Proximal to each partition, the ureter was pinned onto a Sylgard support. The renal and bladder ends of the ureters were connected via pulleys to isotonic transducers for recording of mechanical activity. Electrical stimuli were applied at the R-, M- or B-site by means of field stimulation. Panel a: single electrical pulses, applied at the R-, M- or B-site by field stimulation, induced propagated phasic contractions at both the R- and B-site. Note that the amplitude of evoked contractions was independent of the site of stimulation. Panel b: section of the ureter at the M site excited latent pacemakers to generate a propagated contraction at both the R- and B- site; after section, application of KCl at the M-site likewise produced a propagated wave of contraction at the R- and B- sites. Panel c: after section of the ureter at the M-site, electrical stimuli applied at the R site failed to induce a propagated contraction at the B-site; likewise, electrical stimulation at the B-site failed to induce a propagated contraction at the R-site. Electrical stimulation at the M-site evoked a propagated contraction at both B- and R-site. In all panels, the amplitude of the evoked contractions is expressed as % of the response to 80 mM KCl applied at the R- or B-site.

recorded at this level, as follows: (*a*) approximately 10% of cells ("pacemaker" cells) that display spontaneous activity characterized by a slow rising and repolarizing phases triggered on top of a slowly developing prepotential; (*b*) the majority of cells displayed a more complex, "ureter-like" action potential characterized by a fast spike and a slow long-lasting plateau; (*c*) a third type of action potential was also observed, "intermediate" between those recorded from pacemaker and "driven" cells. Lang and coworkers also reported that cells firing pacemaker potentials have lower resting membrane potential than "driven" cells (-42 and -56 mV, respectively). They observed that nifedipine and cadmium ions blocked all spontaneous activity and depolarized the cell membrane indicating the absolute requirement of L type Ca channels for action potential generation in the renal pelvis. The action potential of both "pacemaker" and "driven" cells was also modulated by potassium (K) conductances, because blockade of Ca-dependent potassium (K) channels by charybdotoxin or apamin increased the duration of action potentials of "driven" cells, whereas other K channel blockers, tetraethylammonium (TEA), or 4-aminopyridine (4-AP), increased the frequency of action potential discharge in both "pacemaker" and "driven" cells (Zhang and Lang, 1994; Lang *et al.*, 1995; Lang and Zhang, 1996).

Zhang and Lang (1994) proposed that the pacemaker action potentials are recorded from the atypical smooth muscle cells identified by Gosling and Dixon (1972, 1974) as the putative pacemaker elements and that the action potentials recorded from "intermediate and "driven" cells originate from smooth muscle cells that are in close and far electrical contact with a pacemaker region, respectively.

In principle, the observed enrichment of pacemaker cells in the proximal renal pelvis may depend on a sampling bias if, for some reason, efficient impalements of pacemaker cells were easier to perform in the proximal versus distal renal pelvis. This interpretation seems unlikely because action potentials exhibiting differing waveforms and closely resembling those recorded from single cells were also obtained by multicellular electrophysiological recordings (sucrose gap technique) from the proximal versus distal guinea pig renal pelvis (Maggi *et al.*, 1995a; Santicioli *et al.*, 1995a; Santicioli and Maggi, 1997). Spontaneous action potentials were discharged from both regions of the renal pelvis, but the frequency of discharge was markedly higher in the proximal renal pelvis where it approached the same frequency values recorded from the intact renal pelvis in organ bath experiments (4.6 versus 1.2 cycles/min in the proximal and distal renal pelvis) (Santicioli and Maggi, 1997). Action potentials recorded from the proximal renal pelvis were bell-shaped, did not show a plateau phase, and had a small after-hyperpolarization although action potentials from the distal renal pelvis had a "ureter-like" morphology (initial spike, long lasting pla-

teau with oscillations) and displayed a sizable after-hyperpolarization. Moreover, the rate of rise of the action potential was markedly faster in the distal than proximal renal pelvis (5.5 $\pm$ 1.3 and 50.9 $\pm$ 15 mV/s, respectively, $n = 65$ and 69, $P < 0.01$, Santicioli P and Maggi CA, unpublished data). Nifedipine (1 μM) suppressed action potentials at both levels and a submaximally effective concentration of nifedipine (50 nM) shortened the action potential duration with similar intensity at both levels. Bay K 8644 prolonged the action potential duration in both regions of the renal pelvis (Santicioli and Maggi, 1997). With regard to K channel blockers, charybdotoxin (30 nM) prolonged the action potential duration at both levels, although 4-aminopyridine exerted a pure positive chronotropic effect in the distal renal pelvis without affecting the resting membrane potential or other parameters of the action potential and was also without effect in the proximal renal pelvis (Santicioli and Maggi, 1997). The K_{ATP} channel opener, cromakalim, determines a glibenclamide-sensitive suppression of spontaneous action potentials in the guinea pig renal pelvis, suggesting the presence of K_{ATP} channels at this level (Maggi *et al.*, 1995a).

2. Action potentials in the ureter. The action potential of the ureter is unusually long lasting and is characterized by an initial spike followed by a plateau (fig. 3). In the guinea pig, the action potential is further characterized by the presence of multiple oscillations on the plateau (Kuriyama *et al.*, 1967; Kuriyama and Tomita, 1970; Shuba, 1977a,b), whereas this phase is not observed in other species (fig. 3). Tetrodotoxin has no effect on the action potential of the guinea-pig ureter (Kuriyama *et al.*, 1967). Washizu (1966) did not report any change of the action potential when extracellular Na was replaced by lithium (Li). Others reported that removal of Na abolished the plateau phase of the action potential (Kuriyama and Tomita, 1970; Shuba, 1977a; Brading *et al.*, 1983). The removal of extracellular Ca totally eliminated the action potential (Kuriyama and Tomita, 1970; Kochemasova, 1971; Brading *et al.*, 1983). Manganese ions (Mn) abolished the initial spike and almost totally eliminated the accompanying contraction. Verapamil markedly depresses the action potential duration and contraction and high concentration of nifedipine almost completely abolished all electrical and mechanical responses (Shuba, 1977a; Brading *et al.*, 1983). The results of these early studies were interpreted to mean that two types of channels are involved in determining depolarization of the smooth muscle of the ureter, a "fast" Ca channel, and a "slow" channel, permeable to both Ca and Na, which would be responsible for the initial spike and for the plateau phase of the action potential, respectively.

The role of K channels in determining repolarization of the smooth muscle of the ureter was appreciated early because the observation that TEA increases the amplitude and duration of the action potential (Shuba, 1977a;
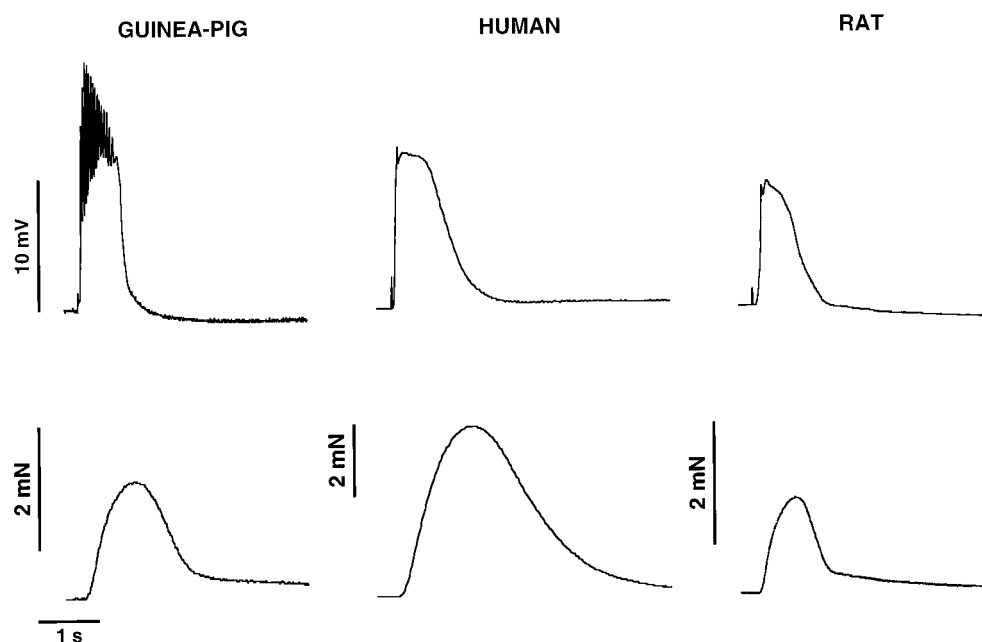
FIG. 3. Action potential and accompanying phasic contraction recorded by sucrose gap from the guinea-pig, human and rat ureter. In each species the action potential is characterized by an initial fast spike followed by a long lasting plateau; in the guinea-pig ureter several oscillations superimpose onto the plateau phase of the action potential.

Brading *et al.*, 1983). The results of pharmacological experiments indicate that in addition to TEA- and charybdotoxin-sensitive K channels, cromakalim- and glibenclamide-sensitive K channels (presumably a class of K_{ATP} channels) also are present in the ureter and that their activation determines hyperpolarization (Maggi *et al.*, 1994b; De Moura and Lemos Neto, 1994; Hernàndez *et al.*, 1997). In the guinea pig ureter, glibenclamide causes a slight but significant prolongation of action potential duration; because several K conductances can be affected by glibenclamide, these findings do not necessarily imply that K_{ATP} channels modulate the action potential of the guinea-pig ureter (Maggi *et al.*, 1994b).

Several studies on the action potential of the ureter were performed before the discovery that application of depolarizing stimuli determines the release of mediators from sensory nerves (see Section V.). Therefore, an indirect influence of neurotransmitters, especially an inhibitory influence of calcitonin gene-related peptide (CGRP) on the action potential, cannot be ruled out totally. This is especially relevant when considering that endogenous CGRP modulates the resting membrane potential, the refractory period, the excitability, and availability of Ca channels in the smooth muscle of the ureter (see Section V.B.). A comparison of parameters used to excite the smooth muscle of the guinea pig ureter in different studies suggests that in vitro capsaicin pretreatment, which blocks CGRP release from sensory nerves, enables the study of the effect of drugs on electromechanical coupling without the confounding factor represented by the concomitant activation of the inhibitory sensory innervation. In particular, a very long width of applied electrical pulses (range 50 msec to 3 sec) has been required to obtain reproducible responses in the intact guinea-pig ureter (Kuriyama and Tomita, 1970; Shuba, 1977a,b; Brading *et al.*, 1983; Bullock and Wray, 1998); this parameter of stimulation can be considerably reduced (1–5 msec) in capsaicin-pretreated preparations (Maggi *et al.*, 1994a,b, 1996a). In vitro capsaicin pretreatment or application of a CGRP receptor antagonist augments the excitability of the smooth muscle of the ureter to electrical stimulation: under these conditions, (fig. 4) the improved stability of electrical recordings makes it easier to perform a thorough quantitative pharmacological analysis of the effects of drugs on the action potentials of the renal pelvis and ureter (Maggi *et al.*, 1994a; 1995a,b; 1996a,b; Santicioli and Maggi, 1997). Further improvement in excitability and stability of recordings can be obtained by omitting magnesium ions from the bathing solution (Patacchini *et al.*, 1998).

By taking advantage of these technical advancements that enabled us to obtain long-term stable recordings of electrical and mechanical activity, we addressed the possible interaction between Ca entering the cell via L-type Ca channels and Ca uptake into the sarcoplasmic reticulum in regulating the action potential and excitation-contraction coupling of the guinea pig ureter. With this aim, we studied the effects of the L-type Ca channel agonist, Bay K 8644, and the blocker of Ca-induced Ca-release from sarcoplasmic reticulum, ryanodine (Missiaen *et al.*, 1992 for review), alone and in combination, on the refractory period, and action potential of the guinea pig ureter. We found that ryanodine exerts various effects on the action potential that include an in-
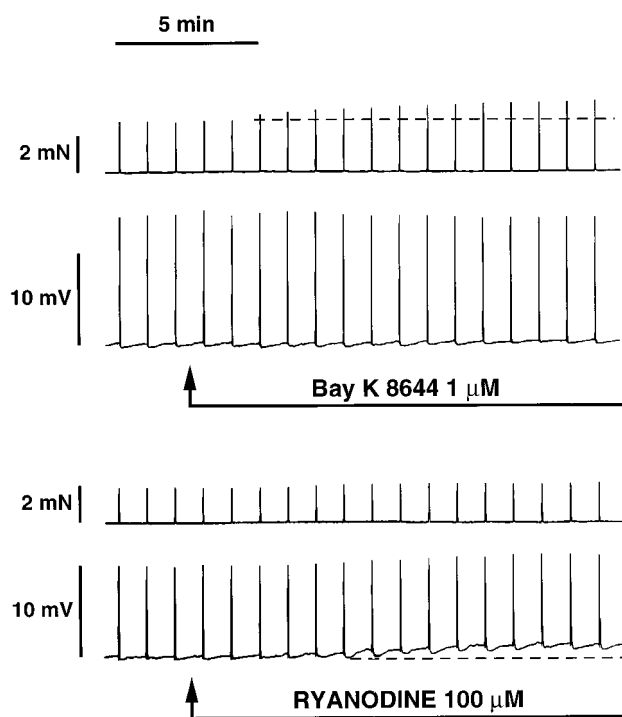
FIG. 4. Electrophysiological recording (lower tracing membrane potential, upper tracing mechanical activity) of the response of the guinea-pig ureter to electrical stimulation using the sucrose gap technique. Electrical stimulation produced action potentials and accompanying phasic contractions: Bay K 8644 (1 μM) enhanced the amplitude of the evoked phasic contractions. Ryanodine (100 μM) produced membrane depolarization without affecting the amplitude of evoked contractions. Reprinted with permission from Maggi *et al.* (1994a).

creased excitability of ureter smooth muscle to depolarizing stimuli (fig. 4), a reduction in the interval between stimulus application and the onset of the action potential, a slight decrease in the action potential duration, and an increase in the after-hyperpolarization (Maggi *et al.*, 1994a). These effects are consistent with a model whereby Ca release from the sarcoplasmic reticulum participates in the setting of the resting membrane potential, probably by activating Ca-dependent K channels. The removal of this mechanism would explain the depolarization observed upon application of ryanodine and also as a consequence, faster achievement of the threshold for firing of the action potential during a depolarization step. A somewhat opposite effect would occur during the Ca load produced by the action potential itself: in this case, a blockade of Ca uptake into the sarcoplasmic reticulum may cause a faster or stronger activation of Ca-dependent K channels, leading to a substantial reduction of action potential duration and an increased after-hyperpolarization (Maggi *et al.*, 1994a).

Importantly, a dissociation between action potential duration and contractility was evidenced in the presence of ryanodine: in fact, the length of the contraction-relaxation cycle was not reduced by ryanodine, despite the shortening of action potential duration. Moreover, a marked additive effect of ryanodine and Bay K 8644 on the duration of action potential and contraction-

relaxation cycle was evidenced (fig. 5), further indicating that Ca reuptake into the sarcoplasmic reticulum has a role in terminating the contractile cycle of the guinea pig ureter.

The characteristics of the action potential have been also studied in freshly dispersed single cells of the guinea pig ureter (Imaizumi *et al.*, 1989a; Lang, 1989): the threshold for initiating an action potential lies between −50 and −40 mV, there is a slow upstroke velocity of 1 V/sec and an overshoot to approximately +30 mv with an action potential duration of approximately 1 sec. In cells with large inward currents oscillations of potential during the plateau phase were recorded (Lang, 1989). Overall, the action potential recorded from single ureteral cells had a remarkable resemblance to that recorded from the intact ureter, although the spikes recorded from the intact tissue are more regular and regenerative than the potential oscillations seen in isolated cells (Lang, 1989).

B. Characterization of Ionic Currents/Channels

1. The calcium current. Lang (1989, 1990) and Imaizumi *et al.* (1989a,b; 1990) showed that voltage-dependent Ca channels provide the main inward current
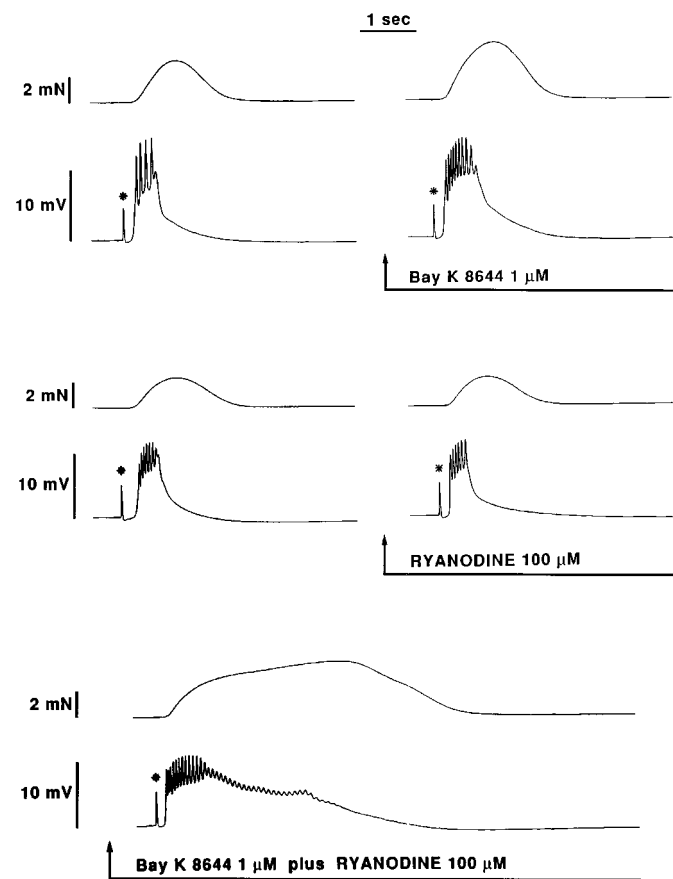
FIG. 5. Effect of Bay K 8644 (1 μM, upper panel), ryanodine (100 μM, middle panel) or Bay K 8644 and ryanodine (1 and 100 μM, respectively, lower panel) on the electrical and mechanical response of the guinea-pig ureter to electrical stimulation. In each panel, the asterisks indicate stimulus artifact. Reprinted with permission from Maggi *et al.* (1994a).

detectable in smooth muscle cells of the guinea pig ureter. The inward Ca current (I_{Ca}) of ureter smooth muscle exhibits a biexponential decay with a duration intermediate between that observed in rapidly spiking smooth muscles (which is remarkably shorter) and that observed in certain weakly spiking vascular smooth muscles (which is remarkably longer) (Lang, 1989).

Imaizumi et al. (1989a) noted several important aspects of I_{Ca} in guinea pig ureter: (a) Ca-induced inactivation of I_{Ca} occurs with a time course much slower than that observed in other smooth muscles; (b) the inactivation does not increase significantly in response to a train of depolarizing pulses; and (c) a very slowly inactivating or noninactivating " window" current can flow through the Ca channels at the plateau potential. Altogether, these observations suggest that the very slowly inactivating I_{Ca} is responsible for the plateau of action potential and that further activation of the current is involved in the generation of the spikes (Imaizumi et al., 1989a). Imaizumi et al. (1989b) measured a non-inactivating I_{Ca}, peaking at approximately 10 mV, and calculated that it may be large enough to explain the sustained tonic contractions observed in response to prolonged depolarization of the guinea pig ureter.

The characteristics of I_{Ca} were further analyzed by Lang (1990) in conditions enabling the exclusion of the interference of time-dependent K currents. Lang (1990) found that the activation of I_{Ca} occurs at potential positive to -40 mV and maximal activation was at $+20$ mV. He also noted, in contrast to other smooth muscles, that the extracellular Ca concentration $[Ca]_o$ does not determine any substantial acceleration of I_{Ca} inactivation in ureteral cells, indicating some peculiarity in the mechanisms of current-dependent inactivation of I_{Ca} in the ureter. Sui and Kao (1997a,b), using Ca rather than Ba as the physiological charge carrier, provided a detailed estimate of the I_{Ca} voltage-current relationship. They concluded that the very slow inactivation of I_{Ca} and the feeble Ca-induced inactivation of I_{Ca} are causally related and contribute significantly to determining the plateau of the action potential of the ureter. According to their estimates, a significant window current allows Ca influx at membrane potentials of -20 to 0 mV, which can help to sustain the plateau phase of the action potential (Sui and Kao, 1997a,b).

2. Potassium (K) currents. Two main outward currents have been characterized in the guinea pig ureter (Lang, 1989; Imaizumi, 1989a, 1990; Sui and Kao, 1997c), a TEA- and charybdotoxin-sensitive Ca-dependent K current or $I_{K(Ca)}$ and a voltage-dependent Ca-insensitive transient K current (also termed A-current or I_{TO}) which is TEA-insensitive but blocked by 4-aminopyridine (4-AP).

$I_{K(Ca)}$ is by far the most important current for determining repolarization and action potential duration. Blockers of $I_{K(Ca)}$ such as TEA or charybdotoxin, produce a marked prolongation of the action potential duration

in ureteral cells. Sui and Kao (1997c) noted that the average outward $I_{K(Ca)}$ in the ureter is very small as compared with that measured in other smooth muscle cells, and that $I_{K(Ca)}$ in ureter cells has a quite low probability of opening in the range of voltage which is achieved during the action potential. According to these authors, the meagerness of $I_{K(Ca)}$ in the presence of a persistent I_{Ca} is central to explain the long plateau of the action potential in ureteral smooth muscle cells.

Sui and Kao (1997c) also proposed that the fluctuations observed on the plateau of the action potential are caused by slight changes in the net membrane current originating from the balance between I_{Ca} and $I_{K(Ca)}$. This interpretation contrasts with that forwarded by Imaizumi et al. (1989a): they proposed that a release of Ca from the sarcoplasmic reticulum would activate the $I_{K(Ca)}$ responsible for transient hyperpolarizations causing the oscillations superimposed on the plateau of the action potential. Imaizumi et al. (1989a) reported that spontaneous transient hyperpolarizations (< 10 mV in amplitude) occur in approximately 60% of ureteral cells. Under voltage clamp conditions, spontaneous transient outward currents (STOCs) were measured, similar to those described in other types of smooth muscle cells (Benham and Bolton, 1986). In ureteral cells, STOCs were first enhanced and then abolished by the application of caffeine, suggesting that they originate from the activation of a Ca-dependent outward current linked to the spontaneous release of Ca from the sarcoplasmic reticulum. According to Imaizumi et al. (1989a), episodic releases of quantal packets of Ca from the sarcoplasmic reticulum would determine STOCs, in turn responsible for the fluctuations observed on the plateau phase of the action potential. In line with this interpretation, Imaizumi et al. (1989a) reported that the multiple oscillations observed on the plateau of the action potential of guinea pig ureter muscle cells were blocked by TEA or caffeine. On the other hand, Sui and Kao (1997c), by using either caffeine or 8-(diethylamino)octyl-3,4,5-trimethoxybenzoate HCl, failed to get evidence for a role of Ca mobilization from the internal store in regulating the fluctuations of the action potential.

We observed that ryanodine, cyclopiazonic acid, and thapsigargin, three drugs which, via different mechanisms, produce a depletion of the sarcoplasmic reticulum Ca store (Maggi et al., 1994a; 1995b; 1996a; Maggi CA and Santicioli P, unpublished data) all produce depolarization of the guinea pig ureter smooth muscle. These observations are consistent with a role of Ca released from the sarcoplasmic reticulum in the setting of membrane potential via activation of Ca-dependent K channels. However, the same three drugs did not abolish the multiple oscillations present on the plateau phase of the action potential of the guinea pig ureter (Maggi et al., 1994a; 1995b), an observation that is against the idea that Ca mobilization from the internal store would be responsible for this phase of the action potential.

The role of the transient K current (I_{TO}) is unclear because it would be rapidly inactivated by depolarization and does not seem to be suited for modulating the shape of the action potential. Because a similar current was shown to regulate interspike intervals in neurons, it was speculated (Lang, 1989; Imaizumi *et al.*, 1990) that, in ureteral cells, I_{TO} may regulate the generation of spontaneous action potentials in response to stretch or that produced in response to excitatory transmitters. I_{TO} may regulate membrane excitability by opposing the Ca current activated around the threshold of the action potential. The selective positive chronotropic effect exerted by 4-AP in the guinea pig proximal renal pelvis may suggest a role of I_{TO} in regulating the frequency of pacemaker potentials (Santicioli and Maggi, 1997).

A remarkable and distinguishing feature of ionic currents recorded from the guinea pig ureter is the *absence* of a 4-AP-sensitive voltage-dependent and Ca-insensitive K current (I_K), which has been recorded in most other smooth muscle cells. As pointed out by Lang (1989), the action potential in most readily spiking smooth muscle cells is kept brief by the sum of the rapid inactivation of the Ca current and a rapidly activating K current which, in part, is Ca-insensitive. I_K is by far the most important outward current in weakly spiking smooth muscle cells: therefore, in the absence of an equivalent I_K, the duration of the action potential is chiefly determined by the inactivation of the Ca current.

$I_{K(Ca)}$ seems to be the major target for the excitatory action of noradrenaline and histamine in the guinea pig ureter (Muraki *et al.*, 1994). Noradrenaline causes a marked inhibition of $I_{K(Ca)}$ which is stronger than its concomitant inhibition of I_{Ca}; by contrast, noradrenaline does not affect I_{TO} (Muraki *et al.*, 1994). By suppressing $I_{K(Ca)}$ noradrenaline causes a marked prolongation of the action potential duration which may account for its excitatory effect on electromechanical coupling of the ureter.

Despite pharmacological evidence for the existence of cromakalim- and glibenclamide-sensitive K channels in the guinea pig renal pelvis and ureter (see Section III.A.), no K_{ATP} current has been characterized thus far in these smooth muscles.

3. Sodium currents. It has been shown that a reduction of extracellular Na ($[Na]_o$) decreases the plateau phase of the action potential of the guinea pig ureter, a finding which was initially interpreted to mean that a "slow" channel, admitting both Na and Ca, could be involved in this phase, whereas a "fast" Ca channel would be responsible mainly for the initial spike (unaffected by Na replacement) (Kuriyama and Tomita, 1970; Shuba, 1977a).

Imaizumi *et al.* (1989a) showed that, in single ureter cells, the replacement of extracellular Na with Li results in a marked acceleration in the decay of I_{Ca}. They excluded the possibility that an increase in outward currents or a faster inactivation of I_{Ca} could be responsible

for this effect and speculated that a late component of the inward current may be dependent upon $[Na]_o$. Because the removal of extracellular Ca totally eliminates the inward current, it seems that an elevation of $[Ca]_i$ is required for establishing the Na-dependent contribution to I_{Ca}. This behavior may involve the activation of an electrogenic Na/Ca exchange (Aickin *et al.*, 1984; 1987; Aaronson and Benham, 1989) or of a Ca-dependent Na current (such as a nonselective cation channel) (Imaizumi *et al.*, 1989a).

In a subsequent study, Muraki *et al.* (1991) reported that two distinct Na currents can be detected in guinea pig ureter muscle cells, one that is fast inactivating (< 10 msec) and sensitive to blockade by tetrodotoxin (EC_{50} 11 nM) and a second one that is slowly inactivating (> 500 msec) and tetrodotoxin-resistant. The fast inactivating current was only observed in approximately 10 to 20% of ureter cells. It was speculated that this current may affect membrane excitability and action potential conduction although stimuli of appropriate strength produce tetrodotoxin-resistant action potentials in the guinea pig ureter.

Lang (1989), by using 7.5 mM Ba as charge carrier, found that the inactivation kinetics of I_{Ca} are not consistently changed by replacement of extracellular Na with TEA. Sui and Kao (1997a) failed to detect any significant contribution of Na to the inward current of ureteral myocytes nor did they observe an effect of Na on currents carried by other ions.

C. Excitation-Contraction Coupling in the Renal Pelvis and Ureter

1. Role of extracellular calcium. Early studies on the characteristics and ionic requirements of the action potential of the guinea pig ureter had demonstrated that extracellular Ca is essential for excitation-contraction coupling and that changes in duration of the action potential are closely paralleled by changes in contractility (see Section III.A.). Indeed, a suppression of action potentials, or a pharmacological modulation (increase or decrease) of its duration by nifedipine, Bay K 8644, or charybdotoxin, are closely paralleled by equivalent changes in the amplitude and duration of spontaneous contractions of the guinea pig renal pelvis (Santicioli and Maggi, 1997).

Several studies have shown that Ca channel blockers suppress both spontaneous and stimulated contractions of the ureter and renal pelvis (Golenhofen and Lammel, 1972; Maggi and Meli, 1984; Hertle and Nawrath, 1984a). However, when studying receptor-mediated contractility, such as noradrenaline-induced contractions, Ca channel blockers preferentially inhibit the phasic but not tonic-type responses (Golenhofen and Hannappel, 1978; Hertle and Nawrath, 1984b). Pharmacologically, studies with agonists and antagonists indicate that L-type but not T-type or N-type Ca channels mediate electromechanical coupling in the smooth muscle of the ure-

ter (Hertle and Nawrath, 1989; Maggi *et al.*, 1994a; Maggi and Giuliani, 1995).

It has been repeatedly noted that organic Ca channel blockers, such as nifedipine, D-600, or verapamil, are more potent in inhibiting the plateau phase of the action potential than the initial spike (Washizu, 1967; Brading *et al.*, 1983; Aickin *et al.*, 1984; Burdyga and Magura, 1986a). By shortening the action potential duration, these drugs markedly depress the phasic contraction accompanying the action potential of guinea pig ureter. These observations support the idea that Ca influx via voltage-dependent channels chiefly determines tension development in the ureter, yet remarkably high concentrations of Ca channel blockers are needed to suppress the initial spike.

The differential sensitivity of the initial spike and of the plateau phase of the action potential to Ca channel blocking drugs is mirrored by the effects of these drugs on changes in membrane potential and tension produced by increasing concentrations of $[K]_o$. Mild elevations of $[K]_o$ determine the firing of action potentials and a concomitant phasic type contractility of the ureter, whereas high $[K]_o$ determines a phasic and tonic contraction sustained by the firing of action potentials and a sustained depolarization (Washizu, 1967; Sunano, 1976; Johnishi and Sunano, 1978). By analogy with their differential effects on the action potential evoked by electrical depolarization, Ca channel blockers are very potent in blocking the tonic component of K-induced contraction whereas high concentrations of Ca channel blockers are needed to suppress the phasic contractions (Sunano, 1976; Brading *et al.*, 1983; Burdyga and Magura, 1986a; Maggi and Giuliani, 1995).

The different potency of nifedipine in inhibiting the phasic and tonic components of the response to high K was found to be a time-dependent phenomenon: in fact, nifedipine inhibited with similar potencies the tonic contraction to 80 mM KCl in guinea pig isolated ureter after 15 and 120 min contact times (EC_{50} 0.67 and 0.29 nM, respectively), whereas its potency in inhibiting the phasic response was increased by approximately 77-fold by prolonging the incubation period (EC_{50} 121 and 1.56 nM after 15 and 120 min contact time, respectively) (Maggi and Giuliani, 1995). These results were interpreted to mean that the L-type Ca channels responsible for generating the phasic component of contraction to high K (presumably the same population of Ca channels responsible for generating the initial spike of action potential) are in a low affinity state for interaction with nifedipine.

In a subsequent study (Maggi *et al.*, 1996b), we showed that different inhibitors of protein kinase A (PKA) suppress the tonic response to high K (fig. 6) at concentrations that have little or no effect on the phasic contractions nor on the action potentials fired in response to electrical stimulation. Contrary to nifedipine, the differential effect of PKA inhibitors (H8 and H89) was not time-dependent; moreover, the effect of H89 was
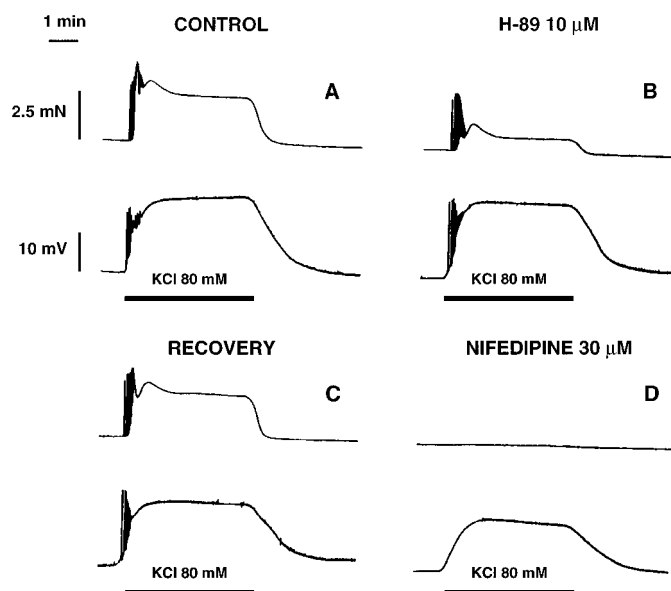

FIG. 6. Changes in membrane potential and tension (lower and upper tracing in each panel, respectively) recorded by single sucrose gap following 5 min superfusion with 80 mM KCl in the guinea-pig ureter (period of KCl application indicated by horizontal bars). Panel A shows the control response: note the appearance of action potentials upon start of superfusion with KCl, followed by a sustained depolarization and tonic contraction. Panel B shows the response obtained after 30 min superfusion with 10 μM H89; the tonic contraction to KCl is markedly depressed by H89; panel C shows recovery from the effect of H89, after 60 min superfusion with drug-free Krebs solution; panel D shows the effect of 30 min superfusion with 30 μM nifedipine. Reprinted with permission from Maggi *et al.* (1996b).

not reproduced by protein kinase C or protein kinase G inhibitors. These results indicated that PKA modulates the availability of a subpopulation of L-type Ca channels, notably those which are in a high affinity state for nifedipine. Because the noninactivating component of I_{ca} is held responsible for the tonic contraction developing in response to prolonged depolarization of the ureter (Imaizumi *et al.*, 1989b), it is possible that phosphorylation by PKA is particularly relevant for the maintenance of the noninactivating state of the Ca channels.

Burdyga and Wray (1997) recently reported the simultaneous recording of action potential, change in intracellular Ca ($[Ca]_i$) and tension development in the rat ureter. They found that approximately 30% of the elevation of $[Ca]_i$ occurred during the upstroke of the action potential whereas approximately 70% of total elevation of $[Ca]_i$ occurred was contributed by the plateau phase of the action potential. The kinetics of tension development and relaxation were significantly slower than those of Ca elevation, the temporal gap likely being covered by phosphorylation/dephosphorylation of myosin light chain.

2. Role of the internal calcium store. Because Ca channel blockers suppress the spontaneous activity of the renal pelvis and the evoked contractility of the ureter, it is evident that an influx of extracellular Ca is crucial for excitation-contraction coupling in the pyeloureteral tract. This knowledge has somewhat delayed the inves-

tigation of the role played by the internal Ca store in excitation-contraction coupling of the ureter, a topic that has recently readdressed.

Some investigators have failed to observe a sizable contractile response to caffeine in the guinea pig ureter and argued that the corresponding intracellular Ca store is insufficient to sustain a contraction (Burdyga and Magura, 1986a; Aaronson and Benham, 1989). Burdyga and Magura (1986b) reported that contractile responses of the guinea pig ureter to caffeine can be elicited by running the experiment at a low temperature (21°C). The observation is consistent with a model in which $[Ca]_i$ elevation produced by caffeine is amplified/prolonged by lowering of temperature in such a way that the $[Ca]_i$ elevation becomes sufficient to evoke a sizable and reproducible increase in tension. However, a transient contraction to caffeine also can be elicited at 37°C in Na-loaded preparations (by exposure to ouabain, see Section III.C.3.) or in preparations depolarized by a high K medium. In these cases, it has been hypothesized that Ca entering in the cell, either via the Na/Ca exchanger operating in a reverse mode (in ouabain-pretreated tissues) or via voltage-dependent Ca channels, had loaded the caffeine-sensitive Ca store (Burdyga and Magura, 1986b).

Imaizumi et al. (1989a) reported that the application of 5 mM caffeine induces contraction of single cells from the guinea pig ureter and transiently enhances spontaneous transient outward currents. Burdyga et al. (1995) observed that the rat and guinea pig ureter show a major difference with regard to the source of intracellular Ca. They found that caffeine (20 mM at room temperature) determines a transient elevation of $[Ca]_i$ and contraction of the guinea pig but not of the rat ureter, whereas caffeine is ineffective in the rat ureter. In contrast, carbachol produced a transient elevation of $[Ca]_i$ and induced a contraction of the rat but not of the guinea pig ureter. Burdyga et al. (1995) also found that ryanodine and cyclopiazonic acid block the responses to caffeine in the guinea pig ureter, whereas only cyclopiazonic acid blocked the mobilization of $[Ca]_i$ in the rat ureter.

It has to be mentioned that, in the above studies, no attempt had been made to prevent the release of sensory neuropeptides (tachykinins and CGRP) from intramural sensory nerves of the rat and guinea pig ureter, therefore, the possible involvement of this factor in the observed results remains unsettled. Moreover, the above studies focussed on the possible role of sarcoplasmic reticulum Ca store in causing a contraction of the ureter smooth muscle, without addressing possible changes in membrane potential.

Working with capsaicin-pretreated guinea pig ureter at 37°C, we found that caffeine (5 mM) causes a glibenclamide-sensitive transient hyperpolarization followed by a sustained depolarization (fig. 7). The hyperpolarization produced by caffeine was blocked by gliben-
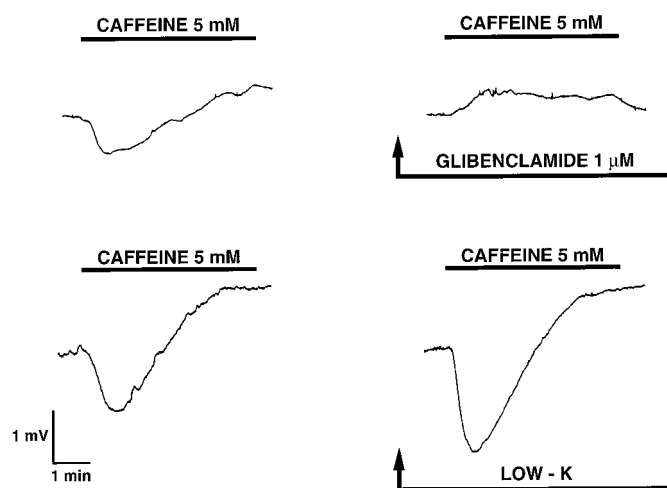

FIG. 7. Recording of membrane potential from the guinea-pig ureter by sucrose gap: application of caffeine produces a transient hyperpolarization which is blocked by 1 μM glibenclamide and enhanced when evoked in a low K (1.2 mM) medium . Reprinted with permission from Maggi et al. (1996a).

clamide, and was reduced when elicited in a Ca-free medium indicating that a Ca-dependent glibenclamide-sensitive K channel is involved in this effect (Maggi et al., 1996a). The role of intracellular Ca stores in regulating the membrane potential of the ureter smooth muscle is also dealt with in Section III.B.2.

Altogether, the results of the above studies indicate that a functional Ca store exists in the mammalian ureter, and that its mobilization can produce various effects on membrane potential, intracellular Ca and tension. The next obvious question relates to the functional role that intracellular Ca plays in excitation-contraction coupling produced by depolarizing stimuli.

To address this question, we studied the effect of ryanodine, cyclopiazonic acid, and thapsigargin on excitation-contraction coupling of the guinea pig ureter (Maggi et al., 1994a; 1995b; 1996a; Maggi CA and Santicioli P, unpublished data). None of these drugs reduced the amplitude of depolarization-induced phasic contractions even after a prolonged contact time: this observation provides clear-cut pharmacological evidence to conclude that Ca release from the sarcoplasmic reticulum does not provide activator Ca for contraction to any significant extent in the guinea pig ureter. However, these drugs produce a transient enhancement of the amplitude of contraction and especially prolonged the duration of the contractile cycle of the ureter (Maggi et al., 1994a, 1995b). These observations imply that uptake of Ca into the sarcoplasmic reticulum is an active mechanism of relaxation in ureteral smooth muscle.

The effect of ryanodine and cyclopiazonic acid also was examined on the biphasic contractile response produced by increasing $[K]_o$ in the guinea pig ureter (Maggi et al., 1994a, 1995b). Both drugs enhanced the phasic contraction to KCl and prolonged the spiking phase on the ascending limb of the developing tonic contractions.

Both effects can be explained on the basis of the ability of these drugs to increase excitability of the ureter, reduce the refractory period for firing of action potentials, and potentiate phasic contractility sustained by action potential generation.

Cyclopiazonic acid, ryanodine, and thapsigargin also produce a transient enhancement of spontaneous contractions of the guinea pig renal pelvis (Maggi et al., 1995a; Santicioli and Maggi, 1997) supporting the idea that Ca reuptake into the internal store modulates the contractile cycle of the renal pelvis. When Ca-induced Ca release from sarcoplasmic reticulum was essential for excitation-contraction, coupling a prolonged exposure to these drugs should produce a suppression or inhibition of spontaneous phasic contractility of the renal pelvis by depleting the Ca store. The failure to observe a depression of contractility even after a prolonged exposure ($>$ 60 min) to these drugs indicates that, similar to the ureter, the sarcoplasmic reticulum does not significantly contribute activator Ca for contraction of the renal pelvis.

3. Sodium/calcium exchange. The existence of a mechanism for Na/Ca exchange in smooth muscle has been a matter of discussion (see Van Breemen et al., 1979 for review). The molecular cloning of different isoforms of the Na/Ca exchanger and analysis of their presence/distribution in various tissues has positively solved the question (see Matsuda et al., 1997 for review), although the definition of the exact role of the exchanger in excitation-contraction coupling of the ureter remains uncertain.

Because the Na/Ca exchange is electrogenic (3 Na are exchanged for 1 Ca) this mechanism may be expected to play a role in regulating the action potential of the ureter. There is evidence which indicates that reducing $[Na]_o$ affects the action potential of the ureter or modifies certain membrane currents in a way that is consistent with the existence of a Na/Ca exchange mechanism (Shuba, 1977a; Imaizumi et al., 1989a). However, other workers have failed to detect a significant contribution of $[Na]_o$ to the inward current and/or modulation of I_{Ca} current in the ureter (Sui and Kao, 1997a).

Some researchers have investigated the properties of the Na/Ca exchanger by focussing on ion distribution measurements. Aickin et al. (1984) showed that after inhibition of the Na/K pump by ouabain, a transient contraction develops in the guinea pig ureter upon Na withdrawal. The contraction developing under these circumstances is dependent upon the intracellular Na concentration ($[Na]_i$) and the presence of extracellular Ca, but is resistant to Ca channel blockers. Direct measurements of $[Na]_i$ (Aickin, 1987; Aickin et al., 1987) indicated that, in the presence of ouabain, $[Na]_i$ stabilizes at a surprisingly low level and that Na could still be extruded against the electrochemical gradient for Na by reducing $[Na]_o$ or elevating $[Ca]_o$. Lamont et al. (1998) recently extended these observations by taking advantage from the use of the Na-sensitive fluorophore, sodium-binding benzofuran isophtalate, to directly measure $[Na]_i$ in guinea pig ureter. They observed that resting $[Na]_i$ is around 10 mM and rose to 25 mM in the presence of ouabain.

The contraction and the concomitant fall in $[Na]_i$ which develop in ouabain-loaded preparations upon removal of $[Na]_o$ were effectively blocked by manganese ions (Mn), a finding consistent with their known blocking activity on the cardiac Na/Ca exchanger (Aickin et al., 1984, 1987). By simultaneously measuring $[Na]_i$, $[Ca]_i$ and tension, Lamont et al. (1998) established the existence of a linear relationship between $[Na]_i$ and developed force in ouabain-pretreated ureters. They also noted that intracellular acidification and alkalinization produced a decrease and an increase, respectively, of the Na withdrawal-induced contraction in ouabain-pretreated ureters and that the changes in developed tension were paralleled by equivalent changes in the $[Ca]_i$ response.

Burdyga and Magura (1988) studied the effect of temperature and Na removal on the relaxation of phasic and tonic contractions in the guinea pig ureter: they reported that the relaxation of the tonic contraction produced by a high K medium was dependent on $[Na]_o$ and speculated that Na/Ca exchange may be involved in terminating tension development in response to this stimulus. Benham and Aaronson (1989) showed that, in the presence of nifedipine, depolarization induced by long pulses of current causes a slow rise of $[Ca]_i$ and activates an outward current that is dependent on the duration and strength of depolarization: the evidence suggests the existence of a Na/Ca exchanger that, in the conditions of recordings, is acting in the reverse mode (Ca entering the cell being exchanged with extrusion of Na). Aaronson and Benham (1989) also showed that the nifedipine-resistant Ca transients were potentiated when lowering the $[Na]_o$, demonstrating that Na/Ca exchange can modulate $[Ca]_i$ when $[Na]_i$ and membrane potential are set or near their physiological levels in ureteral smooth muscle cells. Accordingly, an electrogenic Ca extrusion mechanism during the upstroke of the action potential would result in an inward current that might explain the Na-dependency of the plateau phase of the action potential. In fact, after a Ca load such as that occurring during an action potential, an electrogenic Na/Ca exchange would be expected to mediate a net inward current as though it contributed to extrusion of the Ca load. Although being consistent with a contributory role of the Na/Ca exchanger to transmembrane Ca movements under normal conditions in the guinea pig ureter, these observations also indicated that any role of the exchanger in removing Ca from the cytoplasm is affected by the presence of other Ca-removing pathways.

By using blockers of intracellular Ca handling from the sarcoplasmic reticulum, ryanodine, and cyclopiazonic acid, we presented evidence (Maggi et al., 1994a;

1995b) that Ca reuptake into the internal store modulates the contractile cycle of the guinea pig ureter. Cyclopiazonic acid potentiated the amplitude of contractions sustained by the firing of action potentials, however, the potentiating effect was transient and, during prolonged application of the drug, the amplitude of evoked contractions returned toward the baseline. We speculated that an increase in activity of the Na/Ca exchanger may compensate the blockade of sarcoplasmic reticulum Ca pump function in lowering $[Ca]_i$ (Maggi *et al.*, 1995b). To probe this hypothesis, we studied the effect of cyclopiazonic acid after replacement of 60% $[Na]_o$ with either Li or choline. In both media, the amplitude of the evoked contractions was reduced and the potentiating effect of cyclopiazonic acid became sustained (fig. 8). Both results are consistent with the idea that Na/Ca exchanger could contribute to action potential generation and removal of intracellular Ca in the guinea pig ureter.

4. Role of intracellular pH. Intracellular pH (pH_i) is a fundamental parameter for cell physiology by regulating, among others, enzyme activity and metabolic rate. The mechanisms regulating pH_i maintain this parameter at a level considerably more alkaline than that which could be predicted on the basis of the passive distribution of hydrogen ions (H) (Roos and Boron, 1981).

Aickin (1994a,b) reported that an electroneutral Na/bicarbonate cotransporter is the most important mechanism for the maintenance of pH_i in the guinea pig ureter. An amiloride-sensitive Na/H exchanger also exists, but seems of minor importance in physiological conditions. Both mechanisms extrude acid equivalents to protect the inside of the cell from substantial intrinsic acid loading. A chloride/bicarbonate exchanger is activated during intracellular acidosis to transport acid equivalents inside the cell to restore a more normal pH (Aickin et al., 1994a,b).

Because the pH of urine may vary between 4.5 and 8, other investigators have addressed the question of changes in ureteral smooth muscle excitability/contractility produced by the lowering of pH. Cole *et al.* (1990) showed that intracellular acidification increases the force of contraction of human ureteral smooth muscle in response to depolarizing stimuli. Burdyga *et al.* (1996) analyzed the effects of changing intra- and extracellular pH on $[Ca]_i$, electrical activity, and tension in the guinea pig ureter. They found that lowering pH_i greatly augments the phasic contractions of the ureter, whereas intracellular alkalinization exerts a depressant effect on contractility; both maneuvers determined corresponding changes in the phasic elevations of $[Ca]_i$ which precede each phasic contraction. Changes in action potential duration also were observed on acidification and alkalinization. The action potential duration was correspondingly increased and decreased by lowering and elevating pH_i, respectively (Burdyga *et al.*, 1996). This effect is not ascribable to an effect on Ca current that is affected by
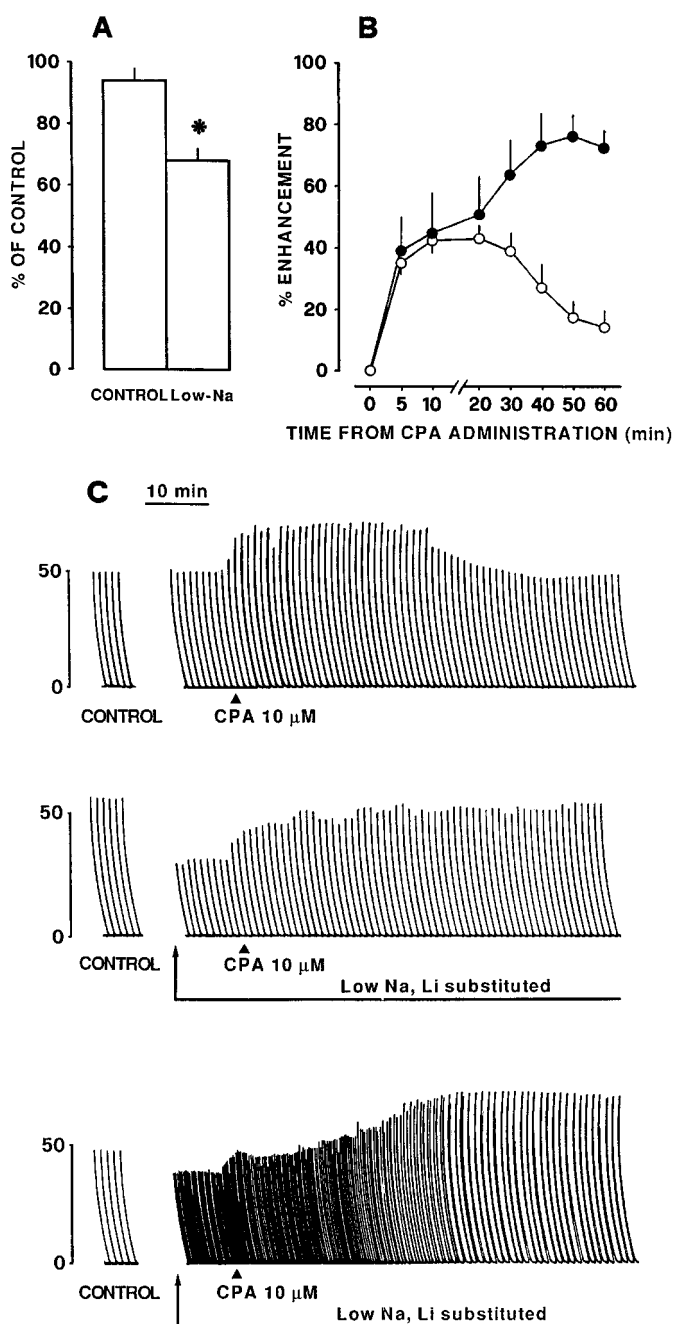
FIG. 8. Panel A: amplitude of EFS-evoked contractions after superfusion with normal Krebs solution (control) or low Na^+, Li^+-substituted Krebs solution in the presence of 1 μM Bay K 8644. Each value is mean ± s.e.m. of 7 experiments. * $P < 0.05$ vs control. Panel B: Effect of CPA (10 μM) on EFS-induced contractions in normal Krebs solution (empty circles) or in low Na^+, Li^+-substituted Krebs solution (filled circles) in the presence of 1 μM Bay K 8644. Each value is mean ± s.e.m. of 7 experiments. Panel C: Typical tracings comparing the effect of CPA on EFS-evoked contractions of the guinea-pig ureter in normal Krebs solution (upper panel) or in low Na^+, Li^+-substituted Krebs solution (middle and low tracings). All experiments were performed in the presence of 1 μM Bay K 8644. Reprinted with permission from Maggi et al. (1995b).

changes of pH_i in the opposite way (increased by alkalinization and decreased by acidification) (Smith *et al.*, 1998). However, the prolongation of action potential duration induced by acidification seems likely ascribable to a blockade of outward K current (Smith *et al.*, 1998).

A decreased force of contraction is usually observed in response to the lowering of pH_i in smooth muscles. Because of the acidity of urine, the unusual response of the ureteral smooth muscle of acidification could be of help by limiting any possible damage from such acidic conditions during the passage of urine. It has to be noted that the lowering of pH is also a very effective stimulus for producing the release of sensory neuropeptides in the lower urinary tract (see Section V.A.).

D. The Refractory Period of the Ureter

As also observed in the heart, a refractory period exists in ureteral smooth muscle; after application of a conditioning stimulus, a time lag of several seconds is required for a test stimulus to induce an electrical and contractile response (Cuthbert, 1965; Kuriyama *et al.*, 1967). The existence of a refractory period may be important for setting the maximum frequency of ureteral peristalsis and can be considered as a kind of safety factor for preventing the occurrence of antiperistaltic waves caused by the excitation of latent pacemakers.

After the discovery of the efferent role played by sensory nerves in the local regulation of pyeloureteral motility, it has been established that the refractory period of the guinea pig ureter depends, at least partially, on the inhibitory action of calcitonin gene-related peptide (CGRP) released from the peripheral endings of sensory nerves by conditioning stimulus (fig. 9; see Section V.). However, even after blockade of the CGRP inhibitory innervation (Maggi and Giuliani, 1994a), a
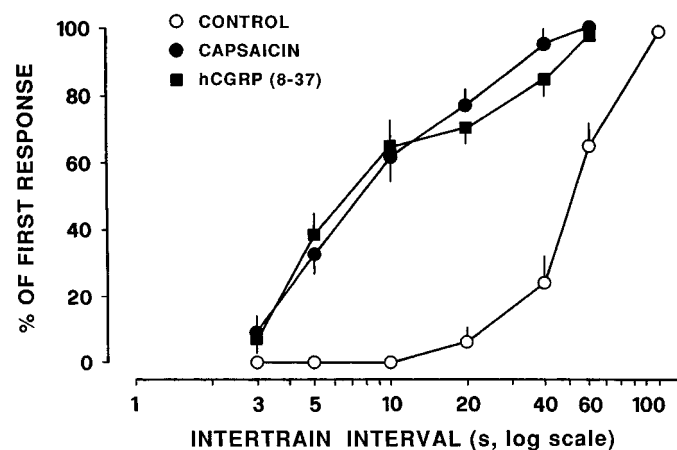
quite long refractory period to applied depolarizing stimuli can be demonstrated, which is myogenic in origin (fig. 9). The use of in vitro capsaicin pretreatment has enabled the study of some factors regulating the myogenic component of the refractory period of the guinea pig ureter after blockade of the inhibitory CGRP innervation (Maggi *et al.*, 1994a, 1995b).

Because voltage-dependent Ca currents undergo Ca-dependent inactivation (Tsien, 1983) and are essential for ureteral excitation and contractility (Sections III.B.1. and III.C.1.), it may be speculated that a reduced availability of Ca channels could be involved in determining the refractory period of the ureter. This hypothesis is supported by the observation (Maggi *et al.*, 1994a) that the L-type Ca channel agonist, Bay K 8644, increases the excitability of guinea pig ureter to depolarization as follows: (*a*) in the presence of Bay K 8644, the refractory period of the ureter was markedly reduced compared with the control; (*b*) in the presence of Bay K 8644, multiple action potentials were evoked during delivery of a prolonged train of stimuli; (*c*) Bay K 8644 did not affect the resting membrane potential; and (*d*) Bay K 8644 did not evoke spontaneous electrical or mechanical activity of the unstimulated ureter. Because the action of Bay K 8644 on L-type Ca channels is not sufficient to produce membrane depolarization of its own, or to activate action potentials, it was concluded (Maggi *et al.*, 1994a) that Bay K 8644 reduces the refractory period of the ureter by increasing the availability of Ca channels. In other words, a decreased availability of L-type Ca channels is one of the mechanisms that regulates the myogenic component of the refractory period of the guinea pig ureter.

Moreover, intracellular Ca, by modulating the resting membrane potential, also contributes to the regulation of the excitability of the smooth muscle of guinea pig ureter. In fact, drugs which deplete the internal Ca store, ryanodine or cyclopiazonic acid, induce a sustained depolarization of the membrane of guinea pig ureter and reduce the refractory period (Maggi *et al.*, 1994a; 1995b). The effect was ascribed to the existence of a "basal" release of Ca from the internal store that regulates the resting membrane potential via activation of Ca-dependent K channels (Imaizumi *et al.*, 1989a). A mild elevation of extracellular K, insufficient to evoke phasic contractions of the ureter, similarly reduces the refractory period of the ureter (Maggi *et al.*, 1994a). Notably, the effect of Bay K 8644 and ryanodine on the excitability of the guinea pig ureter and reduction of the refractory period were additive: in the presence of both drugs, multiple action potentials and phasic contractions were elicited during application of repetitive stimuli at short time intervals (Maggi *et al.*, 1994a).

E. Modulatory Influence of Prostanoids

A role of prostanoids in the local regulation of motility in the upper urinary tract has been postulated. The

FIG. 9. Effect of capsaicin pretreatment or the CGRP receptor antagonist hCGRP (8–37) on the refractory period of the guinea-pig ureter to electrical stimulation. In each preparation a conditioning electrical stimulus was delivered and the duration of the refractory period was quantitated by delivering a second, test stimulus at different time intervals from the conditioning stimulus. The response to the test stimulus, if any was expressed as percent of the amplitude of the response to the conditioning stimulus. Note that control ureter the absolute refractory period exists which exceeds 10 sec; thereafter a response to the test stimulus appeared and an interstrain interval of 100 sec was required to obtain a test response identical with the first one. Both capsaicin and hCGRP (8–37) decreased the refractory period of the guinea-pig ureter indicating that that part of this phenomenon is ascribable to the release of CGRP by sensory nerves during the conditioning stimulus which influences the excitability of the ureter smooth muscle by the test stimulus. Reprinted with permission from Maggi and Giuliani (1994a).

issue is of clinical relevance because cyclooxygenase (COX) inhibitors produce pain relief during renal colic (Holmlund and Sjodin, 1978; Lundstrom *et al.*, 1982; Oosterlink *et al.*, 1990).

Discrepant results have been reported with regard to the effect of prostaglandins on pyeloureteral motility. Prostaglandins of the E or F series produce excitatory effects on the motility of the ureter or renal pelvis (Thulesius and Angelo-Khattar, 1985; Lundstam *et al.*, 1985; Thulesius *et al.*, 1986, 1987; Cole *et al.*, 1988). However, smooth muscle hyperpolarization, relaxation, and cyclic AMP accumulation also have been reported in response to added prostaglandins to the ureter (Johns and Wooster, 1975; Vermue and Den Hertog, 1987). Because different prostanoids can be produced by the same cell type in a species- and stimulus-dependent manner, the results obtained with exogenously added prostaglandins do not necessarily reflect the physiological role of endogenous prostanoids, although the effects produced by COX inhibitors are more informative in this respect.

Several groups have shown that COX inhibitors, e.g., indomethacin, produce a profound inhibitory effect on the spontaneous or evoked motility of isolated pyeloureteral smooth muscles (Thulesius and Angelo-Khattar, 1985; Lundstam *et al.*, 1985; Thulesius *et al.*, 1986, 1987; Cole *et al.*, 1988; Kimoto and Constantinou, 1991). The general conclusion emerging from these studies is that the generation of prostanoids is an important step for the local autocrine/paracrine regulation of pyeloureteral motility. Indeed a complete suppression of pyeloureteral motility by COX inhibitors has been reported in some studies (Lundstam *et al.*, 1985; Thulesius and Angelo-Khattar, 1985; Thulesius *et al.*, 1987; Cole *et al.*, 1988), which suggests that prostanoid generation is mandatory for the maintenance/activation of ureteral peristalsis. The evidence originating from this approach is, however, strictly limited by the selectivity and specificity of the drugs employed. To separate the specific from nonspecific effects of COX inhibitors on pyeloureteral motility, we have compared the effect of the (S)-enantiomer of ketoprofen (active in blocking COX) and of the (R)-enantiomer (inactive in blocking COX) (Hayball *et al.*, 1992) to the effect of indomethacin.

We found that (S)-ketoprofen produced a stereoselective concentration- and time-dependent inhibition of the spontaneous myogenic activity of the guinea pig renal pelvis (Santicioli *et al.*, 1995a). In a sucrose gap, (S)-ketoprofen produced a time-dependent shortening of the duration of spontaneous action potentials of the guinea pig renal pelvis and reduced the amplitude and duration of the accompanying phasic contractions. However, indomethacin totally suppressed the spontaneous activity of the renal pelvis. (S)-ketoprofen had no effect on contractions of the ureter induced by depolarizing electrical pulses nor on those induced by high K, although indomethacin had a marked suppressant effect on both re-

sponses. (S)-ketoprofen had no effect on the propagation of myogenic impulses along the ureter.

Overall, our findings (Santicioli *et al.*, 1995a) demonstrated that stereoselective COX inhibition affects pacemaker potentials and contractility in the guinea pig renal pelvis. The modulatory role of endogenous prostanoids involves an amplification of electromechanical coupling in the renal pelvis although excitability, contractility, or propagation of impulses along the ureter are almost independent of prostanoid generation. Reports of a total suppression of pyeloureteral motility by indomethacin may reflect the additive inhibitory effect exerted by specific COX inhibition and nonspecific effects on electromechanical coupling. Considering the effect of (S)-ketoprofen as a pure consequence of full COX inhibition, it would follow that modulation of action potential duration is the main mechanism through which endogenous prostanoids regulate the pacemaker potentials of the guinea pig renal pelvis. These findings suggest an amplifying or facilitatory effect of endogenous prostanoids on the L-type calcium current which sustains the pacemaker potential of the renal pelvis.

The data presented by Zhang and Lang (1994) further define the modulatory role of endogenous prostanoids in regulating the spontaneous electrical and mechanical activity of the renal pelvis. These investigators showed that indomethacin inhibits the amplitude and frequency of action potentials from " driven" cells in the guinea pig proximal renal pelvis and eventually caused a failure of driven cells to fire action potentials although the underlying pacemaker potentials were unaffected. Zhang and Lang (1994) therefore proposed that endogenous prostanoids facilitate the coupling between pacemaker and driven cells, thereby allowing the spread of electrical activity in the renal pelvis.

The reinforcement of pacemaker activity of the renal pelvis by endogenous prostanoids could be of importance for the effect of COX inhibitors on renal colic. In addition to a possible central analgesic effect, a reduction of renal blood flow and a reduced urine production are held as major mechanisms through which COX inhibitors produce pain relief in renal colic (e.g., Perlmutter *et al.*, 1993). Assuming that endogenous prostanoids reinforce the spontaneous excitation-contraction coupling in the renal pelvis, blockade of this effect by COX inhibitors may contribute to the overall pain relief produced by these drugs in renal colic, by reducing intraureteral pressure.

F. An Integrated View of Myogenic Factors Regulating Excitation-Contraction Coupling in the Renal Pelvis and Ureter

For more than a century, the "myogenic theory" of ureteral peristalsis has shaped investigations on ureteral physiology: great emphasis has been given to hydrodynamic factors, such as the rate of urine flow, in determining the size and pattern of urine boluses which,

in turn, affect the mechanical aspects of rhythm, rate, peristaltic amplitude, and baseline pressure (Kiil and Setekleiv, 1973; Boyarsky and Labay, 1969). Although a neurogenic contribution has been proposed from time to time, there is little doubt that neural factors play, at best, only a modulatory role on ureteral peristalsis.

The current understanding of excitation-contraction coupling in the renal pelvis and ureter has been enriched by the characterization of the ionic currents responsible for changes in electrical activity and by pharmacological studies on the mechanisms of excitation-contraction coupling. The definition of the existence of a functional capsaicin-sensitive innervation (Section E.) and its selective elimination by capsaicin pretreatment have also enabled the study of the myogenic regulation of pyeloureteral motility under more controlled conditions.

In the renal pelvis, the pacemaker activity originates from a subpopulation of specialized cells in the inner muscular layer of renal calyces and pelvis (Gosling and Dixon, 1972, 1974): these cells resemble the interstitial cells of Cajal which are the pacemaker in the smooth muscle of the intestine (Huizinga et $al.$, 1997; Lang et $al.$, 1998). The nature of the "clock" producing the regular firing of spontaneous action potentials by these cells remains to be established.

The spreading of the pacemaker electrical activity through the smooth muscle of the renal pelvis and its coupling with contraction both involve the activation of voltage-dependent L-type Ca channels. These channels also are recruited obligatorily for propagation of ureteral peristalsis and electromechanical coupling in the ureter smooth muscle.

I_{Ca} flowing through L-type Ca channels is viewed as the main (Imaizumi et $al.$, 1989a; Lang, 1989; Sui and Kao, 1997a,b) inward current in ureteral smooth muscle. A noninactivating or slowly inactivating component of I_{Ca} generates a "window" current (Imaizumi et $al.$, 1989b; Sui and Kao, 1997b) that likely is responsible for determining the plateau phase of the action potential, as well as providing a sustained activation of Ca channels during prolonged depolarizing stimuli. Phosphorylation by PKA could be especially important for enabling L-type Ca channels to sustain a prolonged depolarization (Maggi et $al.$, 1996b). L-type Ca channels in the ureter appear to be less prone to Ca-induced inactivation than in other smooth muscles (Sui and Kao, 1997b). This characteristic, and the absence of a voltage-sensitive Ca-independent K current (Imaizumi et $al.$, 1989a; Lang, 1989), seem to be the main factors responsible for the peculiar long duration of the action potential of ureter. Considering the potentiating effects produced by Bay K 8644, charybdotoxin, and TEA, the inactivation of L-type channels and the activation of $I_{K(Ca)}$ are the main factors responsible for terminating the action potential.

The nature of the oscillations observed during the plateau of the action potential of the guinea pig ureter remains controversial (Imaizumi et $al.$, 1989a; Sui and Kao, 1997c). It is possible that the oscillations superimposed onto the plateau of action potential recorded from single cells and from multicellular preparations have a different origin.

A controversy exists in the literature concerning the role of Na currents and the Na/Ca exchanger in regulating the action potential. Several results indicate that a Na/Ca exchanger exists in the ureter, which works in reverse mode (Ca entering the cell) in Na-loaded (ouabain-pretreated) preparations. Considering the effects produced by removing extracellular Na on the action potential, it has been proposed that the Na/Ca exchanger provides part of the depolarizing current which accounts for the long duration of the action potential. However, no direct contribution of extracellular Na to the inward current has been observed (Sui and Kao, 1997a). Functional studies also have indicated that Na/Ca exchange is likely involved in modulating the contraction/relaxation cycle of the guinea pig ureter.

Release of Ca from intracellular store(s) is apparently not essential for providing activator Ca for excitation-contraction coupling but contributes to the setting of the resting membrane potential, probably through the activation of Ca-dependent K channels. Ca reuptake into internal stores is also a mechanism involved in terminating the contractile cycle of the ureter although other mechanisms support this function after blockade of the sarcoplasmic reticulum Ca pump. In particular, three distinct mechanisms could be important (Burdyga and Magura, 1988; Maggi et $al.$, 1994a, 1995b): (a) Ca uptake into sarcoplasmic reticulum that seems especially suited for speeding up the relaxation of phasic contractions; (b) the Na/Ca exchange mechanisms; and (c) a Ca pump at the membrane level; the latter two mechanisms being possibly more important in terminating tonic than phasic-type contractions.

IV. Innervation of the Renal Pelvis and Ureter

A. Sources of Innervation to the Pyeloureteral Tract

The mammalian ureter is innervated mainly by unmyelinated fibers that originate from the renal, ovarian/spermatic, and sympathetic plexuses. The lower part of the ureter may receive a pelvic innervation at least in some species (Wharton, 1932; Saria et $al.$, 1983). The sympathetic supply to the ureter arises from T11-L1 spinal segments. At least part of these fibers synapse in the distal pole of the inferior mesenteric ganglion (Janig and McLachlan, 1987).

The dorsal root ganglia of origin of the afferent innervation of the ureter have been identified by retrograde tracing studies at the level of L2-L3 and S1-S2 in guinea pigs and at the level of T11-L3 and L6-S1 in rats (Su et $al.$, 1986; Semenenko and Cervero, 1992). In guinea pigs, approximately 40% of total labeled cells project to both ureters (Semenenko and Cervero, 1992).

B. Distribution of Nerves

Hoyes et al. (1975a) compared the innervation of the ureter in a variety of species and reported several species-related differences with regard to the frequency of nerve profiles in different layers of the ureter. It appears that the majority of axons terminate in the mucosa and that the innervation of the muscle of the renal pelvis is somewhat larger than that of the ureter (Notley, 1968; Gosling and Dixon, 1971). In humans, the lower ureter receives a denser innervation than the upper ureter, suggesting a major contribution from the pelvis plexus (Edyvane et al., 1992).

In the mucosa, nerve fibers form networks on the luminal aspect of the muscle layer and beneath the basement membrane of the epithelial layer (Dixon and Gosling, 1971; Hoyes et al., 1975b). Varicose nerve terminals originating from the subepithelial layer terminate in the intercellular spaces between the basal cells of transitional epithelium (Notley, 1968; Hoyes et al., 1975b). The existence of a dense mucosal/subepithelial innervation lends support to the hypothesis that the majority of ureteral nerves subserve an afferent function. This is further supported by the notion that pretreatment with toxic doses of capsaicin, causes degeneration in approximately 70 to 85% of axons in the rat or guinea pig ureter (Chung et al., 1985; Kiraly et al., 1991), whereas the subepithelial nerve plexus was resistant to treatment with neurotoxic doses of 6-hydroxydopamine (Hoyes et al., 1975b).

The distribution of afferent nerves to the renal pelvis and ureter also has been assessed directly by the anterograde transport of a tracer injected in rat dorsal root ganglia T12-L1 (Marfurt and Echtenkamp, 1991). In the ureter, the afferent innervation is densest in the proximal part and decreases caudally: labeled fibers were observed in all sectional layers of the ureter (adventitia, smooth muscle, subepithelium, and epithelium). The highest density of afferent innervation was seen in the renal pelvis with a similar distribution to that described in the ureter.

C. Ureteric Ganglia

The existence and distribution of ureteric ganglia is a controversial topic: Elbadawi and Schenk (1969) reported the presence of ganglia in the lower two-thirds of the cat ureter. Beatty and Gabella (1988) and Mitchell et al. (1993) reported the presence of ganglia and individual neurons along the entire length of the guinea pig ureter. Some studies have failed to detect periureteral ganglia (Notley, 1968; Dixon and Gosling, 1971) although in other studies, the presence of ganglia at the uretero-vesical junction in several species has been noted (Gosling, 1970; Wharton et al., 1981). Periadventitial ureteric ganglia are numerous in the chicken ureter (Sann et al., 1992, 1997) and are thought to modulate ureteral peristalsis in this species.

D. Cholinergic Nerves

Cholinergic nerves are present in the pyeloureteral tract of several species (Schulmann, 1985; Wharton et al., 1981; Prieto et al., 1994). Acetylcholinesterase-positive (AChE$^+$) nerves are present in the adventitia, smooth muscle, and mucosa of cat and guinea pig ureter, along with periadventitial AChE$^+$ positive ganglion cells in the distal part (Wharton et al., 1981). In the intravesical equine ureter, AChE$^+$ nerve fibers distribute along blood vessels and smooth muscle and form a subepithelial plexus in the ureteral mucosa. AChE$^+$ neurons are present also in adventitial small ganglia, which suggests that part of the cholinergic innervation is derived from this local source (Prieto et al., 1994). The density of AChE$^+$ nerve fibers increases from the pelvic end of the equine ureter to the bladder: the intravesical region is the most densely innervated (Prieto et al., 1994). Sann et al. (1995a) recently reported that a substantial part of cholinergic nerve fibers in the subepithelial plexus of the rat ureter are sensory in origin. They found that nerve profiles staining positively for choline acetyl transferase immunoreactivity colocalize with CGRP-like immunoreactivity.

In organ bath experiments, acetylcholine increased the contractile activity (tone/phasic activity) of the pig intravesical ureter (Hernàndez et al., 1993), the sheep uretero-vesical junction (Rivera et al., 1992), the guinea pig renal pelvis (Maggi and Giuliani, 1992), and the equine ureter (Prieto et al., 1994). In the equine intravesical ureter, acetylcholine also determined a potent endothelium-dependent relaxation of precontracted arteries, via NO generation (Prieto et al., 1994). In the dog, i.v. acetylcholine increased the peristaltic frequency and decreased the volume of urine boluses but atropine had no significant effect on peristalsis (Morita et al., 1987).

There have been reports of contractile responses produced by electrical stimulation of intramural nerves sensitive to blockade by anticholinergic drugs in the guinea pig renal pelvis (Yoshida and Kuga, 1980) and reports of significant release of acetylcholine from human, guinea pig and rabbit isolated pyeloureteral tract in response to nerve stimulation (Del Tacca, 1978). However, we have been unable to demonstrate any significant atropine-sensitive local motor response to nerve stimulation in the guinea pig isolated renal pelvis or ureter (Maggi et al., 1986; Maggi and Giuliani, 1992, 1995). Atropine did not affect spontaneous activity of rabbit isolated renal pelvis nor responses to electrical stimulation of intramural nerves in this species (Del Tacca et al., 1974, 1981).

E. Noradrenergic Nerves

Postganglionic noradrenergic nerves have been demonstrated in all areas of the ureter of several species (Duarte-Escalante et al., 1969; Elbadawi and Schenk, 1969; Wharton et al., 1981; Schulmann, 1985; Prieto et al., 1993). These nerves are commonly observed along

the adventitia and are associated with blood vessels, in both muscle and submucosal layers. Occasional fibers distribute within the smooth muscle. In the rat ureter, dopamine-β-hydroxylase positive (DBH$^+$) nerves provide a dense periarteriolar innervation (Sann *et al.*, 1995b) that colocalizes with NPY-like immunoreactivity. Some DBH$^+$ neurons are present in periureteric ganglia in guinea pigs (Mitchell *et al.*, 1993).

Tyrosine hydroxylase-positive (TH$^+$) nerves distribute to the smooth muscle and around arteries/arterioles of the human ureter, also in the submucosal layer (Edyvane *et al.*, 1992). TH$^+$ nerves colocalize with NPY and account for approximately 50% of total nerve profiles in the human ureter (Edyvane *et al.*, 1994).

Noradrenaline can either stimulate or inhibit renal pelvis and ureteral contractility via α- and β-adrenoceptors, respectively (Hannappel and Golenhofen, 1974b; Del Tacca *et al.*, 1981; Morita *et al.*, 1987; Hernàndez *et al.*, 1992). β-Adrenoceptor stimulation resulted in cAMP accumulation in the guinea pig ureter (Wheeler *et al.*, 1986). A positive inotropic effect of isoproterenol was described in the more proximal part of rabbit renal pelvis (Morita, 1986). In dogs, i.v. noradrenaline caused an increase in peristaltic frequency, an elevation of intraureteral pressure, and a decrease in bolus volume with a resultant decrease in the rate of fluid transport; isoproterenol decreased peristaltic frequency and eventually suppresses ureteral peristalsis (Morita *et al.*, 1987).

Application of guanethidine or that of phentolamine plus propranolol did not affect the local motor responses produced by stimulation of intramural nerves in the rat or guinea pig isolated renal pelvis or ureter (Maggi and Giuliani, 1992; Maggi CA, unpublished data). However, the contractile responses produced by electrical field stimulation in the rabbit renal pelvis seems to be mediated by catecholamines acting via α-adrenoceptors (Gosling and Waas, 1971; Del Tacca *et al.*, 1974).

F. Tachykinins and Calcitonin Gene-Related Peptide

Two tachykinins with an established status of neurotransmitters, substance *P* (SP) and neurokinin A (NKA), and CGRP, are present in primary afferent nerves distributing to the mammalian renal pelvis and ureter. SP/NKA are produced from alternatively spliced forms of preprotachykinin I gene mRNA encoding for both peptides (see Maggi *et al.*, 1993 for review). CGRP also coexists with SP/NKA in many sensory nerves; CGRP positive (CGRP$^+$) nerves exist in the ureter that do not colocalize with SP/NKA (Alm *et al.*, 1978; Hokfelt *et al.*, 1978; Sikri *et al.*, 1981; Hua *et al.*, 1986a,b; 1987; Su *et al.*, 1986; Sann *et al.*, 1992, 1995b; Zheng and Lawson, 1997).

The cells of origin of the SP/NKA/CGRP$^+$ and CGRP$^+$ nerves present in the mammalian upper urinary tract reside in dorsal root ganglia (Semenenko and Cervero, 1992). The peptides synthesized at the level of the neuronal somata are then exported to the periphery. In the guinea pig, most of the DRG neurons (approximately 90%) innervating the ureter are immunoreactive for SP or CGRP and approximately 65% are immunoreactive for both peptides (Semenenko and Cervero, 1992).

The distribution of SP/NKA/CGRP$^+$ nerve fibers to the mammalian ureter has been detailed in several studies (Su *et al.*, 1986; Tamaki *et al.*, 1992; Sann *et al.*, 1995b; Zheng and Lawson, 1997). In the renal pelvis, the fibers run parallel to the long axis of each of the circular and longitudinal muscle layers, resulting in a lattice-like appearance of the nerve fibers. In the ureter, the fibers accumulate in the subepithelial plexus, around blood vessels, and in the muscle layer. An important feature of the SP/NKA/CGRP$^+$ nerves is their sub- and intraurothelial distribution. This location may enable the sensory nerves to detect a backflow of urine into the renal pelvis and ureteral wall: the density of fibers penetrating the urothelium seems larger in the renal pelvis than in the ureter (Zheng and Lawson, 1997).

SP/CGRP$^+$ nerves are also present in the chicken ureter and likely represent sensory innervation also in this species, although sensory neurons in avians are not capsaicin-sensitive (Sann *et al.*, 1992; 1997). In the chicken ureter, SP/CGRP$^+$ nerves distribute mainly in the submucosa and around periureteric ganglia.

In the human ureter SP/CGRP$^+$ nerves were occasionally seen in the smooth muscle but their density is lower than that seen in other species. SP/CGRP$^+$ nerves are mostly present around blood vessels and in the submucosa of the ureter but are scarce in the smooth muscle. SP/CGRP$^+$ nerves account for approximately 4% and 17% of total nerve profiles in the lower and upper ureter, respectively, and a further 5% of nerve profiles is CGRP$^+$ at both levels (Tainio *et al.*, 1991; Edyvane *et al.*, 1992, 1994).

G. Neuropeptide Y

NPY$^+$ nerves have been repeatedly described in the ureter of several species (Allen *et al.*, 1990; Edyvane *et al.*, 1992; Prieto *et al.*, 1997). Their density is markedly species dependent: NPY coexists with noradrenaline in some sympathetic nerves that show a perivascular localization in the rat ureter, also surrounding arterioles in the submucosa. NPY$^+$ nerves in the rat ureter are sensitive to 6-hydroxydopamine treatment, indicating their noradrenergic origin (Allen *et al.*, 1990). The density of NPY$^+$ nerves markedly increases after the administration of neurotoxic doses of capsaicin. This effect could be explained by a competition existing between sensory and noradrenergic nerves for the availability of trophic factors produced by the innervated tissue that promote neuron growth/survival (Sann *et al.*, 1995b). Some NPY$^+$ neurons were reported in guinea pig periureteric ganglia (Mitchell *et al.*, 1993). Prieto *et al.* (1997) described a gradient in the density of NPY$^+$ nerves in the equine ureter, which is maximal at the level of the intravesical region. This region is proposed to act as a

functional sphincter by facilitating urine discharge during bladder filling and by preventing vesico-ureteral reflux during micturition. Prieto *et al.* (1997) described the presence of numerous NPY$^+$ nerves in the smooth muscle, around arteries, and within adventitial ganglia of the intravesical equine ureter. The latter also were found to contain some NPY$^+$ neuronal bodies, therefore, the NPY innervation could have a local origin.

NPY$^+$ nerves are seen in the smooth muscle and around all blood vessels but not in the epithelium of the human ureter (Tainio *et al.*, 1991). Edyvane *et al.* (1992) reported that a vast majority (approximately 80%) of intramural nerves in the human ureter are NPY$^+$. The number of NPY$^+$ nerves is greater than that of noradrenergic nerves, which suggests the existence of a separate population of nonadrenergic NPY$^+$ neurons. The same group subsequently showed that approximately 50% of total nerve profiles in the human ureter colocalizes TH and NPY, although a further 30% of nerve profiles colocalizes NPY and VIP but not TH (Edyvane *et al.*, 1994). Both NPY/VIP$^+$ and NPY/TH$^+$ nerves distribute around blood vessels but the two populations differ with regard to their distribution to the inner and outer muscle layers, respectively (Edyvane *et al.*, 1994).

Exogenously added NPY did not affect muscle tone of the isolated equine ureter, but markedly potentiated the contractile responses to noradrenaline, an effect apparently mediated via Y$_2$ receptors. NPY produced concentration-dependent vasoconstriction of ureteral resistance arteries and potentiated the response to noradrenaline via Y$_1$ receptors (Prieto *et al.*, 1997). Contrary to its effect in other organs, NPY does not modulate the release of sensory neuropeptides from peripheral endings of sensory nerves in guinea pig ureter (Maggi and Giuliani, 1995).

H. Vasoactive Intestinal Polypeptide

VIP positive (VIP$^+$) nerves have been detected in all layers of the cat and guinea pig ureter, and are more frequently observed in the former species (Wharton *et al.*, 1981). A small number of VIP$^+$ periadventitial ganglion cells also was observed in cats (Wharton *et al.*, 1981; Mitchell *et al.*, 1993). VIP$^+$ nerve fibers are present in all layers of the rat ureter and show a distinct regional distribution, but are absent in the renal pelvis and upper ureter, appearing in the middle ureter and then remaining constant in the lower part of the ureter (Sann *et al.*, 1995b). VIP$^+$ nerves in the rat ureter are unaffected by capsaicin pretreatment and, owing to their distribution, were thought to originate from VIP$^+$ neurons in the pelvic ganglion (Sann *et al.*, 1995b).

VIP$^+$ nerves are seen also in the smooth muscle, around blood vessels, and adjacent to the epithelium in the human ureter: approximately 40% of all nerve bundles in human ureter are VIP$^+$ (Tainio *et al.*, 1991; Edyvane *et al.*, 1992). The major part of VIP$^+$ nerves are also NPY$^+$ and form a population distinct from the NPY/TH$^+$ nerves (Edyvane *et al.*, 1994; 1995).

I. Nitroxergic Nerves

Nerve profiles displaying immunoreactivity for nitric oxide synthase (NOS$^+$) or NADPH diaphorase (NADPH$^+$) have been described in the human (Smet *et al.*, 1994; Goessl *et al.*, 1995; Iselin *et al.*, 1997), pig (Hernàndez *et al.*, 1995; Iselin *et al.*, 1997), and sheep ureter (Garcìa-Pascual *et al.*, 1996). NOS/NADPH$^+$ nerves distribute to the smooth muscle, around arteries, and in the subepithelial layers. NOS$^+$ nerves colocalize with both VIP and NPY but never with TH in the human ureter (Smet *et al.*, 1994). NADPH$^+$ neurons are present also in ureterovesical ganglia in humans (Grozdanovic and Baumgarten, 1996).

NO or NO donors relaxed the smooth muscle of the pyeloureteral tract from several species and this effect appeared to be linked to an elevation of intracellular cGMP levels (Iselin *et al.*, 1996; 1997).

However, NO synthase blockers apparently did not affect the motility of the guinea pig isolated renal pelvis or ureter (Maggi *et al.*, 1995a, c) or that of the sheep ureter (Garcìa-Pascual *et al.*, 1996). NO synthase inhibitors blocked the nonadrenergic noncholinergic nerve-mediated relaxations of the pig intravesical ureter, which implies a transmitter role of NO or of an NO-like substance at this level in this species (Hernàndez *et al.*, 1995). In the latter preparation, glibenclamide, but not charybdotoxin or apamin, blocked in parallel the relaxant effect of exogenous NO and that of the endogenous nonadrenergic noncholinergic transmitter, which indicates that endogenous NO acts by opening glibenclamide-sensitive K$_{ATP}$ channels (Hernàndez *et al.*, 1997).

J. Efferent Roles of Nerves in the Pyeloureteral Tract

As seen in this section, there is clear evidence that the mammalian renal pelvis and ureter receive innervation from several sources and that diverse transmitters play a role in the pyeloureteral tract. The neurotransmitter role of sensory neuropeptides, SP, NKA, and CGRP will be extensively dealt with in Section V. Regarding more conventional transmitters, acetylcholine and noradrenaline, the available evidence for their participation to the local regulation of pyeloureteral motility is, at best, a minor one. In particular, a few studies have suggested a role of noradrenaline as an excitatory transmitter in the rabbit renal pelvis (Section IV.E.) but this role does not seem to extend to other species. Based on their observed pattern of distribution, it appears likely that noradrenergic nerves play a role in regulating blood flow in the ureter. Regarding acetylcholine, the results of some studies suggest a transmitter role in the guinea pig ureter (Section IV.D.) but this claim has not been confirmed nor extended to other species. However, there is evidence that NO or a related substance could exert a

neurotransmitter role in the pig intravesical ureter (Section IV.I.).

Therefore, with the exception of the role exerted by sensory neuropeptides and possibly NO, the overall evidence indicates that several transmitters which are clearly important for local regulation of motility in various viscera play a relatively minor role in regulating pyeloureteral motility in mammals.

V. Sensory Neuropeptides in the Pyeloureteral Complex: Release, Actions, and Receptors

A. Adequate Stimuli and Mechanisms Regulating the Release of Sensory Neuropeptides

Because of the dense innervation and high content of sensory neuropeptides, the guinea pig renal pelvis and ureter have been used extensively as test objects to investigate the mechanisms of release of sensory neuropeptides (SP, NKA, and CGRP). Saria *et al.* (1983) first reported that the application of capsaicin causes the release of SP from the guinea pig ureter, a finding confirmed in several subsequent studies and extended to the demonstration that depolarizing stimuli, including electrical stimulation of sensory nerves, also induce the concomitant release of NKA and CGRP in the guinea pig and rat ureter (Hua *et al.*, 1986a; Amann *et al.*, 1988a; Santicioli *et al.*, 1988; Dray *et al.*, 1989; Maggi *et al.*, 1990, 1992b,c). In addition to capsaicin and electrical stimuli, chemical agents, such as bradykinin and bacterial peptides, are effective stimulants of the release of sensory neuropeptides in the ureter and renal pelvis (Maggi *et al.*, 1992a).

The release of sensory neuropeptides from the peripheral endings of capsaicin-sensitive primary afferent nerves is Ca dependent. Some stimuli, including depolarization by electrical pulses, use N-type voltage-dependent Ca channel for inducing secretion of sensory neuropeptides, because the response was inhibited by ω-conotoxin fraction GVIA (Maggi *et al.*, 1990; Maggi and Giuliani, 1991). The release process is also induced by α-latrotoxin, a component of black widow spider venom: this finding implies the involvement of the high affinity α-latrotoxin receptor, neurexin Ia, in the secretion of sensory neuropeptides from afferent nerve terminals (Waterman and Maggi, 1995). However, the degree of utilization of N-type Ca channels for secretion of neuropeptides from ureteral afferent nerves is species-dependent: in the rat ureter CGRP release induced by electrical stimulation is not blocked by ω-conotoxin fraction GVIA (Maggi and Giuliani, 1991). Other stimuli, including capsaicin, produce the release of sensory neuropeptides via a mechanisms that is Ca dependent but does not require the activation of voltage-dependent Ca channels (Maggi, 1995 for review).

B. Actions and Neurotransmitter Role of Sensory Neuropeptides in the Pyeloureteral Tract

The tachykinins, SP and NKA, are powerful stimulants of pyeloureteral motility: in the renal pelvis this

effect is evident as a positive chrono- and inotropic response, producing a marked potentiation of phasic contractility and, at high concentrations, an elevation of tone (Maggi *et al.*, 1992c,d). In the guinea pig renal pelvis, both NK_1 and NK_2 receptors mediate the inotropic and chronotropic effects of tachykinins (Maggi *et al.*, 1992d). When applied to the isolated ureter, which is electrically and mechanically quiescent, tachykinins induced the appearance of phasic contractions sustained by the firing of action potentials, not dissimilar from those evoked by direct electrical stimulation of smooth muscle cells (Hua *et al.*, 1986a,b; Maggi *et al.*, 1986; 1987a; 1988a; Amann *et al.*, 1988). In the guinea pig and human ureter, the application of NKA produced a nifedipine-resistant depolarization onto which a series of nifedipine-sensitive action potentials were superimposed (Patacchini *et al.*, 1998). In the ureter from both species, a series of phasic contractions was produced by NKA in parallel to the evoked action potentials, and these phasic contractions were suppressed by nifedipine. In the human ureter, a nifedipine-resistant tonic contraction also was evidenced (Patacchini *et al.*, 1998).

In sharp contrast with the contractile effect of tachykinins, CGRP inhibits the motility of the isolated renal pelvis and ureter (Hua *et al.*, 1986a,b; 1987; Maggi *et al.*, 1987b). The effect of CGRP is especially evident in the ureter as a suppression of evoked motility: the all-or-none suppressant effect occurs because CGRP abolishes the firing of action potentials evoked either by electrical stimulation of chemical agents (see next section). A descending gradient exists in the guinea pig pyeloureteral tract regarding sensitivity to the inhibitory effect of CGRP: the ureter is extremely sensitive (Maggi *et al.*, 1987b; Maggi and Giuliani, 1991), whereas the spontaneous activity of the renal pelvis is inhibited but not suppressed by this peptide (Maggi *et al.*, 1992c). The sensitivity to CGRP appears in the distal region of the renal pelvis although the motility of the proximal renal pelvis is substantially unaffected by CGRP (Maggi *et al.*, 1995a). Moreover, although the suppressant effect of CGRP in the ureter is largely linked to the activation of glibenclamide-sensitive K channels (see Section V.C.), the inhibitory effect of CGRP in the renal pelvis is glibenclamide resistant (Maggi *et al.*, 1995a). However, cromakalim exerts a glibenclamide-sensitive suppressant effect in both the renal pelvis and ureter; therefore, part of the gradient in sensitivity to the inhibitory effect of CGRP seems to be linked to a differential coupling of CGRP receptors with distinct effector mechanisms.

In the ureter, CGRP totally suppresses the propagation of impulses and, in this way, prevents ureteral peristalsis and suppresses antiperistalsis generated by stimulation of latent pacemakers (Meini *et al.*, 1995).

Sann *et al.* (1992) analyzed the distribution of SP and CGRP receptors in the guinea pig ureter: the SP binding sites (NK_1 receptor) were associated with blood vessels and the epithelium with the density order:

venules > epithelium > arterioles; although CGRP binding sites were chiefly distributed on smooth muscle. This distribution agrees with the notion that tachykinin NK_1 receptors mainly mediate inflammatory reactions initiated by the stimulation of afferent nerves (Section V.D.), although CGRP mainly mediates the concomitant changes in smooth muscle activity. In the chicken ureter SP binding sites also are prominent in neurons of ureteric ganglia (Sann et al., 1992; 1997). Applied SP evoked the depolarization of a subset of neurons in the chicken ureteric ganglia and desensitization to SP reduced the responsiveness of these neurons to mechanical stimuli, suggesting that locally released SP may play a role in modulating neuronal activity in this species (Sann et al., 1997).

Peptidases limit the extent and duration of action of tachykinins and CGRP in the pyeloureteral tract: in the renal pelvis, the application of peptidase inhibitors potentiated the contractile effect of exogenous and endogenous tachykinins (Maggi et al., 1992d). In the guinea pig ureter, the inhibitory action of CGRP was reduced because of degradation by a thiorphan-sensitive peptidase, presumably neutral endopeptidase (Maggi and Giuliani, 1994b).

Since the early studies on this topic, tachykinins and CGRP have been proposed to act as transmitters producing a local modulation of motility in the pyeloureteral tract. Hua et al. (1986b) reported that the i.v. injection of capsaicin inhibits ureteric motility in anesthetized guinea pigs when administered at a low dose, whereas a biphasic response (inhibition followed by excitation) was produced when capsaicin was administered at a high dose. They also showed that i.v. administered CGRP produces inhibition although i.v. injected NKA produces excitation of ureteric motility. Hua et al. (1986b) speculated that the dual effect produced by a high dose of capsaicin may involve a differential release of these neuropeptides. Hua et al. (1986b) also showed that electrical stimulation of the inferior mesenteric ganglion produces a dual effect on ureteric motility similar to that exerted by a high dose of i.v. capsaicin. These findings were interpreted as an indication that transmitters released from sensory nerves can affect ureteral motility in vivo, probably through a local effect in the ureteral wall (Hua et al., 1986b). The evidence that sensory nerves do indeed exert a *local* control of ureteral motility by producing transmitter release in the ureter wall was provided by an independent study (Maggi et al., 1986). After having induced the appearance of a background phasic motility, we found that electrical field stimulation (EFS) of intramural nerves or bath application of capsaicin produced a transient inhibitory effect of the motility of the rat isolated ureter by suppressing the activity of latent pacemakers (Maggi et al., 1986). The response to EFS was abolished by tetrodotoxin, by pretreatment with high doses of capsaicin or by cold denervation, whereas it was unaffected by chronic bilateral

removal of the pelvic ganglia (Maggi et al., 1986; 1987a). Overall, these data indicated that capsaicin exerts its effect in the rat ureter through a subset of intramural sensory nerves which are activated antidromically by EFS to release an inhibitory mediator, which was later identified as CGRP (Maggi et al., 1987b).

Amann et al. (1988a) showed that tachykinin and CGRP-like immunoreactivity are present in approximately similar amounts in the guinea pig ureter, whereas CGRP levels are approximately 33-fold higher than tachykinin levels in the rat ureter. Moreover, the application of capsaicin produced the corelease of tachykinin and CGRP-like immunoreactive material from guinea pig isolated ureter whereas only the release of CGRP-like immunoreactivity was detected from the rat iso lated ureter (Amann et al., 1988a). Under comparable in vitro conditions, Amann et al. (1988a) showed that the application of capsaicin exerts both excitation of latent pacemakers (ascribable to release of tachykinins) and suppression of NKA-activated latent pacemakers (ascribable to release of CGRP) in the guinea pig isolated ureter, whereas only an inhibitory effect could be demonstrated in that rat isolated ureter. Overall, the findings of Amann et al. (1988a) established that the different quality of the local response to capsaicin observed in the rat and guinea pig isolated ureter represents a true species-related difference, likely linked to the different ratios of CGRP and tachykinins stored and released from sensory nerve terminals.

A local control of motility by neuropeptides (tachykinins and CGRP) released from the peripheral endings of capsaicin-sensitive sensory nerves also was demonstrated in the guinea pig renal pelvis (Maggi and Giuliani, 1992; Maggi et al., 1992c; Patacchini et al., 1998): the prevailing motor response produced by capsaicin or EFS is the induction of a long lasting positive chronoand inotropic effect that is tachykinin mediated. After blockade of the excitatory action of tachykinins, an unopposed transient inhibitory effect, CGRP-mediated, was disclosed.

The development of suitable receptor antagonists has enabled the conclusive identification of tachykinins and CGRP as the mediators responsible for the excitatory and inhibitory effects, respectively, observed upon stimulation of sensory nerves in the pyeloureteral tract (see Maggi, 1995 for review). In particular, tachykinin receptor antagonists blocked the local excitatory motor response produced by sensory nerve stimulation in the renal pelvis (Maggi et al., 1992c,d). The CGRP receptor antagonist, CGRP (8–37), was used to prove the involvement of endogenous CGRP in the inhibition of renal pelvis motility observed after blockade of tachykinin receptors (Maggi et al., 1992c) and in the transient suppression of latent pacemakers produced by nerve simulation in the rat and guinea pig isolated ureter (Maggi and Giuliani, 1991).

The local changes in motility produced by stimulating sensory nerves in the renal pelvis and ureter are sustained by changes in the electrical properties of smooth muscle cells. In the ureter, electrical stimulation of intramural nerves determined a transient tetrodotoxin-sensitive hyperpolarization or inhibitory junction potential (i.j.p.) (Santicioli and Maggi, 1994; fig. 10) whose latency, amplitude and duration are frequency-dependent. The i.j.p. was magnified when evoked in low-K medium and was blocked by a CGRP receptor antagonist. The acute capsaicin application determined a transient hyperpolarization of ureteral smooth muscle. The blocker of K_{ATP} channels, glibenclamide also blocked the EFS-induced i.j.p. indicating that endogenous CGRP acts as a K channel opener in producing its transmitter action (Santicioli and Maggi, 1994). However, EFS caused membrane depolarization and increased the frequency of action potential discharge in "pacemaker" cells and prolongation of action potential duration and reduced afterhyperpolarization in "driven" cells of the guinea pig renal pelvis (Lang *et al.*, 1995). In nifedipine-arrested cells of the renal pelvis, nerve stimulation produced a tetrodotoxin-sensitive excitatory junction potential (Lang *et al.*, 1995), which is presumably tachykinin mediated.

Overall, these findings demonstrate that tachykinins and CGRP fulfill all the classical criteria required to establish the neurotransmitter role of a putative medi-
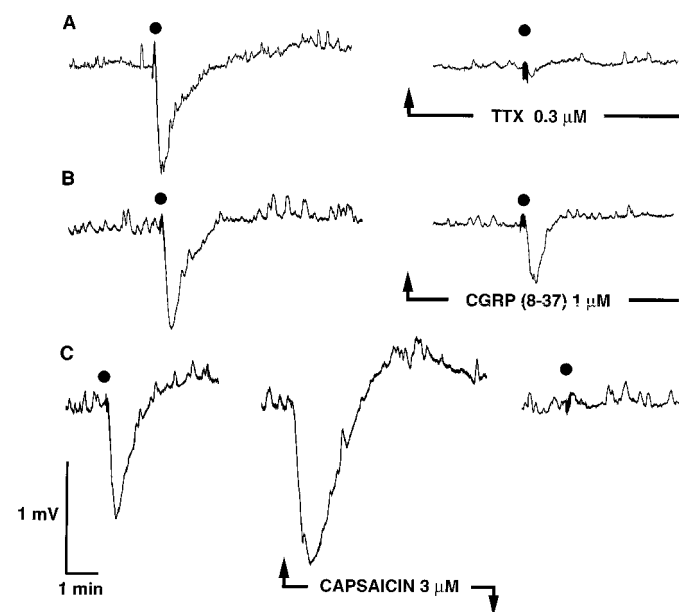

FIG. 10. Recording of membrane potential from the guinea-pig ureter by ucrose gap: application of electrical pulses (5 Hz for 10 sec, 0.1 ms pulse width) by field stimulation determines an inhibitory junction potential (i.j.p.) mediated by CGRP released from the peripheral endings of capsaicin-sensitive primary afferent neurons. The EFS-evoked i.j.p. is abolished by tetrodotoxin (TTX, panel A) and reduced by the CGRP receptor antagonist, CGRP (8–37) (panel B). In panel C, the application of capsaicin determines a prompt and large hyperpolarization: after application of capsaicin (3 μM for 15 min before washout) the EFS -evoked i.j.p. is abolished because of desensitization of primary afferent nerves. Reprinted with permission from Santicioli and Maggi (1994).

ator. The source of the released transmitters (primary afferent nerves) poses interesting limits to the physiological and pathophysiological significance of this process, which is further complicated by the existence of regional variations whereby the action of tachykinins or that of CGRP becomes more apparent when not prevalent. The significance of this transmitter role in integrated models of ureteral peristalsis is not understood yet. A role for CGRP in the local modulation of spontaneous activity of the guinea pig renal pelvis has been recently proposed: Teele and Lang (1996, 1998) reported that application of a local stretch to the proximal renal pelvis determines an inhibitory effect on the contractility of the distal renal pelvis which is partially prevented by the CGRP receptor antagonist, CGRP (8–37), or by glibenclamide, which suggests a role of endogenous CGRP in the modulation of migrating contractions in the renal pelvis.

C. Mechanisms of the Inhibitory Action of CGRP

From the evidence reviewed in the previous sections, it seems that CGRP is the main mediator involved in the local regulation of ureteral motility: its main effect can be described as a powerful suppression of latent pacemakers of the ureter smooth muscle. Three mechanisms, not necessarily unrelated to each other, have been described to account for the smooth muscle relaxant activity of CGRP: (*a*) stimulation of adenylyl cyclase and elevation of intracellular cAMP; (*b*) generation of nitric oxide (NO); and, (*c*) activation of glibenclamide-sensitive potassium (K) channels (see Poyner, 1992 for review).

On this background, we have studied the mechanism of the inhibitory action of CGRP in the guinea pig ureter: to eliminate the confounding influence of endogenous CGRP from the experimental setup, our studies were performed in capsaicin-pretreated ureters, to block CGRP release from sensory nerves.

In organ bath experiments, regular phasic contractions of the ureter (twitches) (fig. 11) were obtained by applying EFS with pulses of sufficient intensity to excite latent pacemakers thus producing action potentials and accompanying twitches (Maggi and Giuliani, 1994a; Maggi *et al.*, 1994a,b,c; 1995c; 1996a,c). When applied cumulatively, CGRP produced a concentration-dependent inhibition of the EFS-evoked twitches with an EC_{50} of 3.3 nM (Maggi *et al.*, 1994c). The NO synthase inhibitor, L-nitroarginine, did not affect twitches or prevent the action of CGRP (Maggi *et al.*, 1994c). Moreover, CGRP did not elevate cGMP levels in the ureter (Santicioli *et al.*, 1995b). Therefore, the NO/cGMP pathway cannot be held responsible for the action of CGRP. However, glibenclamide produced a profound, albeit incomplete, inhibition of the relaxant effect of CGRP (Maggi *et al.*, 1994c). When tested on the cumulative concentration-response curve to CGRP, glibenclamide produced a pure depression of the E_{max} to CGRP without determining any significant rightward shift of the curve. This
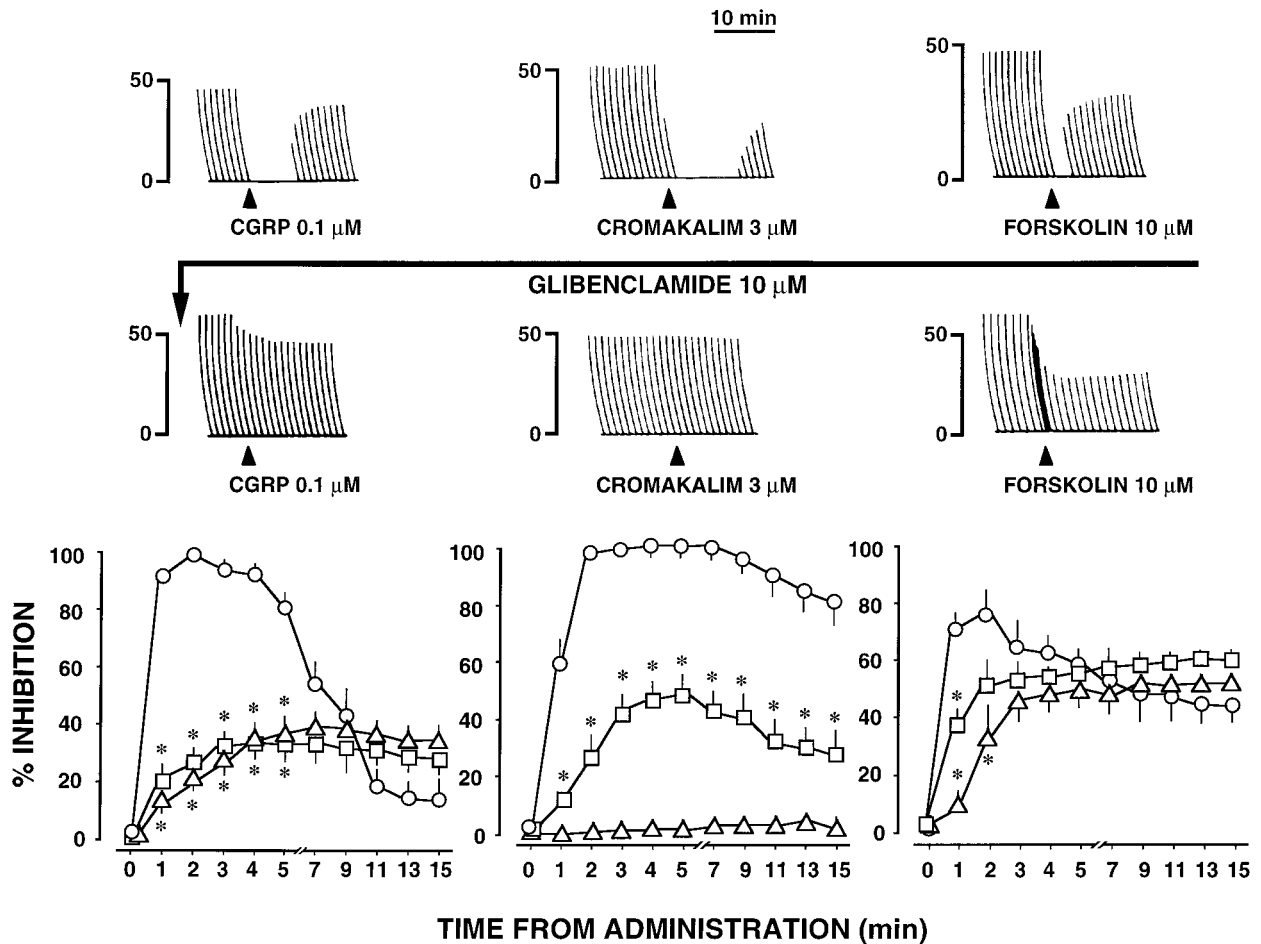
FIG. 11. Tracings (upper and middle panels) and quantitative data (lower panels) showing the time course of the inhibitory effect produced by maximally effective concentrations of CGRP (0.1 μM), cromakalim (3 μM) and forskolin (10 μM) in the absence and presence of glibenclamide (1 and 10 μM). Upper panels shows the control response to the three agonists in the absence of glibenclamide. Middle panels shows the response obtained in paired ureters from the same animals in the presence of 10 μM glibenclamide. Vertical bars are % of the response to KCl. Lower panel shows the response to CGRP (left), cromakalim (middle) and forskolin (right) in the absence (circles) and presence of 1 μM (squares) or 10 μM (triangles) glibenclamide. Each value is mean ± s.e.m. of 6–16 observations.*significantly different from control values, $P < 0.05$. Reprinted with permission from Maggi et al. (1995c).

indicates that glibenclamide blocks an effector mechanism (K channels) downstream to CGRP receptor occupancy (Maggi et al., 1994c). Both 1 and 10 μM concentrations of glibenclamide were equally effective in reducing the E_{max} of the inhibitory effect of CGRP (Maggi et al., 1994c): therefore, a distinct fraction (approximately 35%) of the effect of CGRP is glibenclamide-resistant.

The existence of two distinct components in the relaxant action of CGRP, operationally defined as glibenclamide sensitive and glibenclamide resistant, was further demonstrated in experiments in which a single maximally effective concentration of CGRP (0.1 μM) was applied to the bath (fig. 11; Maggi et al., 1995c, 1996a,c; Maggi and Giuliani, 1994b). These experiments demonstrated that the efficiency of the mechanism of action of CGRP in inhibiting twitches is markedly different: in fact, in the absence of glibenclamide, CGRP produced a prompt and transient total suppression of twitches which recovered with an amplitude lower than that observed before CGRP application (fig. 11). The suppressant effect of CGRP on twitches was abolished by glibenclamide: in the presence of glibenclamide, a slowly developing partial inhibitory effect occurred in response to CGRP (approximately 30% inhibition of twitch amplitude) (Maggi et al., 1995c, 1996a; fig. 11). Notably, under comparable experimental conditions, glibenclamide totally eliminates the suppressant effect of the K_{ATP} channel opener, cromakalim (Maggi et al., 1995c; fig. 11).

The glibenclamide-sensitive suppressant effect of CGRP was influenced by several variables as follows: (a) when applying electrical stimuli of threshold intensity, very low concentrations of CGRP (1 to 3 nM) were sufficient to produce a transient suppression of evoked contractility (Maggi and Giuliani, 1994a), whereas low concentrations of CGRP did not suppress twitches evoked by suprathreshold stimuli (Maggi et al., 1994c); (b) the transient nature of the early suppressant effect of CGRP was not ascribable to receptor tachyphylaxis and partly involved CGRP degradation by peptidases, via a thior-

phan-sensitive mechanism (Maggi and Giuliani, 1994b); however, (*c*) even in the presence of peptidase inhibitors, twitch suppression by CGRP was inherently transient (Maggi *et al.*, 1994b); and, (*d*) upon recovery from suppression, the amplitude of twitches stabilized to approximately 60 to 70% of their original amplitude; the recovery occurred in an all-or-none manner (fig. 11).

The effect of CGRP and cromakalim on the biphasic contractile response induced by elevating $[K]_o$ was also investigated (Maggi *et al.*, 1994c; 1995c). KCl (80 mM) caused a biphasic contraction of the guinea pig ureter, both of which components involved the activation of L-type Ca channels (see Section III.C.1.) but can be pharmacologically differentiated on the basis of their differential sensitivities to Ca channel blockers and PKA inhibitors. CGRP exerted a profound inhibitory action on both components of the response to KCl, whereas cromakalim selectively suppressed the phasic response without affecting the tonic component of contraction (Maggi *et al.*, 1994c, 1995c). Moreover glibenclamide effectively prevented the ability of CGRP (and cromakalim) to suppress the phasic contraction to KCl without affecting the inhibitory effect of CGRP on the tonic contraction to KCl (Maggi *et al.*, 1994c).

These observations further support the concept that the glibenclamide-sensitive component of CGRP action is especially effective in producing a transient suppression of phasic contractions of the ureter, which are sustained by the firing of action potentials. The glibenclamide-resistant mechanism of CGRP action has a limited efficacy in inhibiting phasic contractions but can exert a profound suppression of tonic contractions sustained by prolonged depolarization.

1. CGRP, hyperpolarization and blockade of phasic contractions. The efficiency of CGRP action in producing a total suppression of phasic contractions of the ureter suggested an indirect effect on smooth muscle contractility. CGRP produced a glibenclamide-sensitive hyperpolarization of the smooth muscle of guinea pig ureter (Santicioli and Maggi, 1994) which closely resembles the hyperpolarizing and action of cromakalim (Maggi *et al.*, 1994b). By producing hyperpolarization, CGRP prevented the firing of action potentials to depolarizing stimuli (fig. 12). In the presence of glibenclamide, CGRP shortened the action potential duration and reduced the amplitude of the accompanying contraction, but the hyperpolarization and the consequent suppressant effect were abolished (Maggi *et al.*, 1994a). A glibenclamide-sensitive hyperpolarization was produced also by endogenous CGRP (Santicioli and Maggi, 1994) and this effect is capable to suppress action potentials and twitches produced by chemical stimulation of latent pacemakers (Maggi *et al.*, 1987b; Maggi and Giuliani, 1991).

The nature of K channels activated by CGRP has been deduced indirectly, on the basis of pharmacological data. The sensitivity of the inhibitory effect of CGRP to glibenclamide (IC_{50} 0.13 μM) and to low concentrations of
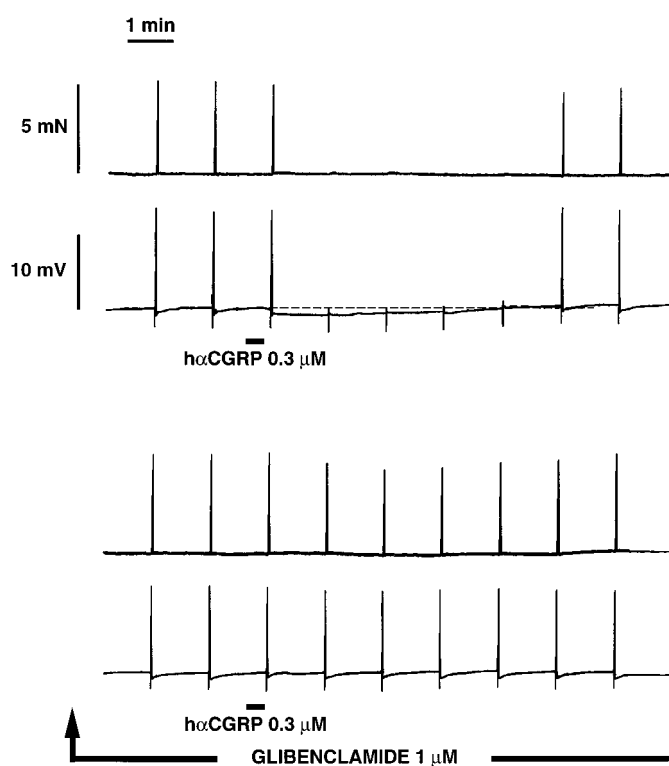

FIG. 12. Action potential (recorded by sucrose gap technique) and phasic contraction produced by electrical stimulation of the guinea-pig ureter: note the hyperpolarization and transient suppression of evoked electrical and mechanical responses produced by CGRP (0.3 μM for 15 sec, applied at bars) and antagonism of the action of CGRP by glibenclamide. Note that in presence of glibenclamide CGRP produced a small inhibition of the amplitude of evoked contractions. Reprinted with permission from Maggi *et al.* (1994c).

barium ions (IC_{50} 63 μM) both support the involvement of a class of K_{ATP} channels. This conclusion is further supported by the observation that apamin and charybdotoxin did not affect the CGRP-induced hyperpolarization (Maggi *et al.*, 1996a).

2. CGRP and cAMP accumulation. In arterial and gallbladder smooth muscle, CGRP activates a class of K_{ATP} channels via cAMP accumulation and stimulation of PKA (Quayle *et al.*, 1994; Zhang *et al.*, 1994a,b; fig. 13A). Therefore we addressed the questions: (*a*) does CGRP stimulate adenylyl cyclase to produce cAMP accumulation in the guinea pig ureter and (*b*) is this effect linked, possibly via stimulation of PKA, to activation of K channels?

Direct measurements indicated that CGRP determines cAMP accumulation in the guinea pig ureter, as forskolin also does, whereas cromakalim is ineffective in this respect (Santicioli *et al.*, 1995b). Moreover, glibenclamide did not affect the cAMP elevation induced by CGRP, which implies that, when cAMP elevation is causally related to CGRP hyperpolarization, the activation of K channels occurs downstream to stimulation of adenylyl cyclase.

A series of pharmacological experiments was undertaken to verify the causal relationship between cAMP
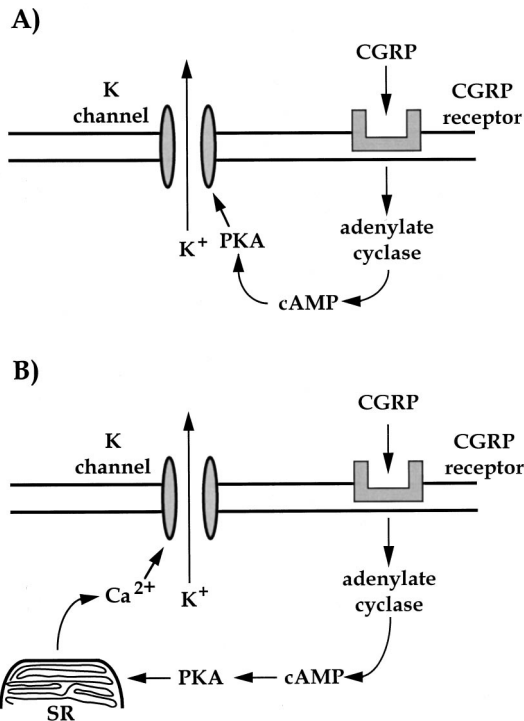
A)

B)

FIG. 13. Schematic drawing illustrating the proposed chain of events linking the occupancy of CGRP receptors to activation of K channels in the guinea-pig ureter (see text for details). According to the available data, CGRP would activate adenylyl cyclase and produce cAMP accumulation in ureter smooth muscle cells which, in turn, activates protein kinase A (PKA). PKA could directly phosphorylate glibenclamide-sensitive K channel leading to hyperpolarization (scheme A). This chain of events has been proposed as being responsible for K channel activation by CGRP in other smooth muscles. However several considerations, detailed in the text argue against a plain relationship between cAMP accumulation and K channel activation in the guinea-pig ureter. Moreover, pharmacological evidence indicates that mobilization of Ca from the sarcoplasmic reticulum is involved in the action of CGRP: blockers of sarcoplasmic reticulum Ca mobilization prevent CGRP-induced acivation of K channels in the same manner as PKA inhibitors or cAMP antagonist do. From this evidence, reviewed in the text, an alternative scheme is proposed whereby PKA activated by cAMP elevation determines Ca release from the sarcoplasmic reticulum, producing activation of a class of Ca-sensitive K channels blocked by glibenclamide (scheme B).

elevation and K channel activation in the hyperpolarizing response to CGRP. In particular, the effect of several drugs which interfere with cAMP accumulation/action via PKA was investigated: (a) stable cAMP analogs; (b) forskolin, which directly activates adenylyl cyclase; (c) the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX); (d) the stereoselective cAMP antagonist, Rp-cAMPS and; (e) PKA inhibitors, the isoquinoline derivatives H8 and H89.

On the "agonists side" both forskolin (fig. 11) and IBMX produced a transient glibenclamide-sensitive suppression of twitches, thus mimicking the response to CGRP (Maggi et al., 1995c). Moreover, forskolin caused a glibenclamide-sensitive hyperpolarization of the membrane of the ureter (Santicioli et al., 1995b). Similar to its effect on CGRP, glibenclamide totally eliminated the twitch suppressant effect of forskolin, although not affecting a later and sustained partial inhibitory effect on twitch amplitude (Maggi et al., 1995c). It seems, therefore, that forskolin mimics both the glibenclamide-sensitive and the glibenclamide-resistant components of CGRP action.

An involvement of cAMP in the CGRP-induced hyperpolarization was further suggested by the results of experiments with "antagonists." Both Rp-cAMPS, a stereoselective competitive antagonist of cAMP at its binding site on PKA (Van Haastert et al., 1984; Rothermel and Parker Botelho, 1988) and the isoquinoline derivatives, H8 and H89, which block PKA by acting as ATP antagonists on the catalytic subunit of the kinase (Hagiwara et al., 1987), prevented twitch inhibition by CGRP in the absence but not in the presence of glibenclamide (Maggi et al., 1995c). In other words, PKA inhibitors blocked the glibenclamide-sensitive component of CGRP action without affecting the glibenclamide-resistant response. H89 is approximately 10-fold more potent inhibitor of PKA than H8 (see Hidaka and Kobayashi, 1992 for review) and a similar potency ratio was found for inhibiting the glibenclamide-sensitive component of CGRP action. Moreover, neither Rp-cAMPS nor H8 or H89 prevented the suppressant effect of cromakalim although they blocked the early, glibenclamide-sensitive response to both forskolin and IBMX (Maggi et al., 1995c).

Summarizing, the results obtained with forskolin, IBMX, Rp-cAMPS, H8, and H89 support the idea that PKA activation might link the cAMP elevation produced by occupancy of CGRP receptors and the activation of glibenclamide-sensitive K channels. Several studies have supported an involvement of PKA in maintaining the activity of K_{ATP} channels via channel phosphorylation (Ribalet et al., 1989; Nakayama et al., 1991; Quayle et al., 1994) and the same mechanism may underlie K channel activation by CGRP in the ureter. However, three sets of data cannot be easily fitted into a simple signaling pathway (fig. 13A) involving CGRP receptor stimulation -> adenylyl cyclase activation and cAMP elevation $\rightarrow$ PKA activation $\rightarrow$ phosphorylation and opening of K^+ channels. These are as follows:

1. First, membrane-permeable and metabolically stable cAMP analogs failed to reproduce the glibenclamide-sensitive early suppressant action of CGRP, forskolin, and IBMX (Maggi et al., 1995c). A lack of mimicry of putative cAMP-mediated relaxations by cAMP analogues has been repeatedly described (see Murray, 1990 for review). The failure of cAMP analogs to reproduce the profound and transient glibenclamide-sensitive inhibition of twitches may reflect the importance of a kinetic factor in the action of CGRP, forskolin and IBMX. A common element to the latter agents is that cAMP levels are elevated *within* the cells, either after membrane receptor occupancy (CGRP), direct adenylyl cyclase stimulation (forskolin) or prevention of cAMP degradation (IBMX). It may be suggested

that membrane permeation by the cAMP analogues is too slow to reproduce a brisk elevation of intracellular cAMP (and PKA activation), a factor that may be required for activation of K_{ATP} channels. The existence of a kinetic factor in the action of CGRP is further suggested by the transient nature of the suppressant effect produced by these agents, because the twitch amplitude recovered, after a transient suppression, despite the continued presence of forskolin (fig. 11) and IBMX (Maggi et al., 1995c).

2. The second set of "discrepant" data arises from a kinetic comparison of the inhibitory effect of CGRP (or forskolin) versus their ability to elevate cAMP levels. Both CGRP and forskolin produced a prompt hyperpolarization of the membrane (Santicioli and Maggi, 1994; Santicioli et al., 1995b) and this " quick" effect matched the time-course of the glibenclamide-sensitive suppression of twitches. However, although an elevation of cAMP was detected at 2 min after the application of CGRP or forskolin, cAMP levels further increased with time reaching a plateau approximately 15 min after administration of either drug (Santicioli et al., 1995b). Therefore, when cAMP elevation is causally related to K channel activation along the scheme depicted in fig. 13, it seems necessary to postulate the existence of a " compensatory" mechanism that limits the duration K channel activation despite the progressive elevation of cAMP levels induced by CGRP and forskolin. One possibility is that the production of cAMP causes a localized decrease in ATP concentrations thus activating K_{ATP} channels (see also Section V.C.4.); such a mechanism could account for the transient nature of K^+ channel activation by CGRP due to diffusion of ATP from the larger volume of the cytosol into the submembrane space.

3. The third set of "discrepant" data arises from a quantitative comparison of the elevation of cAMP levels (Santicioli et al., 1995b) versus the amplitude/duration of the hyperpolarization produced by CGRP and forskolin. Forskolin was more effective in elevating cAMP than CGRP (Santicioli et al., 1995b), yet CGRP was more effective in producing a glibenclamide-sensitive inhibition of twitches (Maggi et al., 1995c). This discrepancy may have several explanations: (a) different compartmentalization of increased intracellular cAMP produced by CGRP and forskolin; (b) a K channel blocker action of forskolin itself (Krause et al., 1988; Inoue et al., 1993), auto-limiting the extent/duration of the induced hyperpolarization; (c) the existence of a depolarizing component in the action of forskolin, which was unmasked in the presence of 10 μM glibenclamide (Santicioli et al., 1995b); and (d) contrary to forskolin, which directly activates adenylyl cyclase, the action of CGRP is likely to be mediated

by a G-protein coupled to CGRP receptors (see Poyner, 1992 for review); a facilitatory influence of G-proteins on K_{ATP} channel activation through an antagonism of ATP-dependent gating (Ito et al., 1994; Terzic et al., 1994) may be postulated.

The results reviewed in this section provide pharmacological evidence that cAMP elevation and PKA activation are causally linked to K channel activation by CGRP in the guinea pig ureter. On the other hand, some elements do not fit into a simple scheme (fig. 13A) whereby cAMP elevation and PKA activation are linearly related to K channel activation. The transient nature of the glibenclamide-sensitive component of CGRP action, as opposed to the sustained character of its glibenclamide-resistant action, indicate the existence of other factor(s), which limit the duration of K channel activation by CGRP.

3. Role of intracellular calcium in the action of calcitonin gene-related peptide. We next became interested in the possibility that mobilization/reuptake of Ca from the sarcoplasmic reticulum may be involved in causing the transient nature of K channel activation by CGRP. In fact, the sarcoplasmic reticulum Ca ATPase is a known target for cAMP-dependent phosphorylation by PKA. PKA can either increase Ca mobilization by, for example, phosphorylating the Ca-induced Ca release channel (Coronado et al., 1994 for review) or, by phosphorylating the sarcoplasmic reticulum protein, phospholamban. PKA can increase the efficiency of the pump in removing free intracellular Ca (see Suematsu et al., 1984; Komori and Bolton, 1989; Murray, 1990 for review). Moreover, the results of our studies had indicated that the sarcoplasmic reticulum may be involved in regulating the resting membrane potential of the guinea pig ureter (see Section III.C.).

In a series of experiments we found that cyclopiazonic acid (fig. 14), ryanodine, and thapsigargin, all prevented the twitch-suppressant effect of CGRP in the absence of glibenclamide, whereas the same drugs did not affect the suppressant effect of cromakalim or the partial inhibition of twitches produced by CGRP in the presence of glibenclamide (Maggi et al., 1996a). In other words, the spectrum of the inhibitory action produced by these three drugs is superimposable to that observed with Rp-cAMPS and with the PKA inhibitors, H8 and H89 (Maggi et al., 1995c). Moreover, we found that cyclopiazonic acid eliminates the membrane hyperpolarization induced by CGRP without affecting that exerted by cromakalim (Maggi et al., 1996a).

These results indicate that the chain of events initiated by adenylyl cyclase activation (direct or receptor-mediated in the case of forskolin and CGRP, respectively) leading to the opening of K channels via cAMP accumulation (Santicioli et al., 1995b), involves a step related to Ca release from the sarcoplasmic reticulum. However, an involvement of classical Ca-dependent K
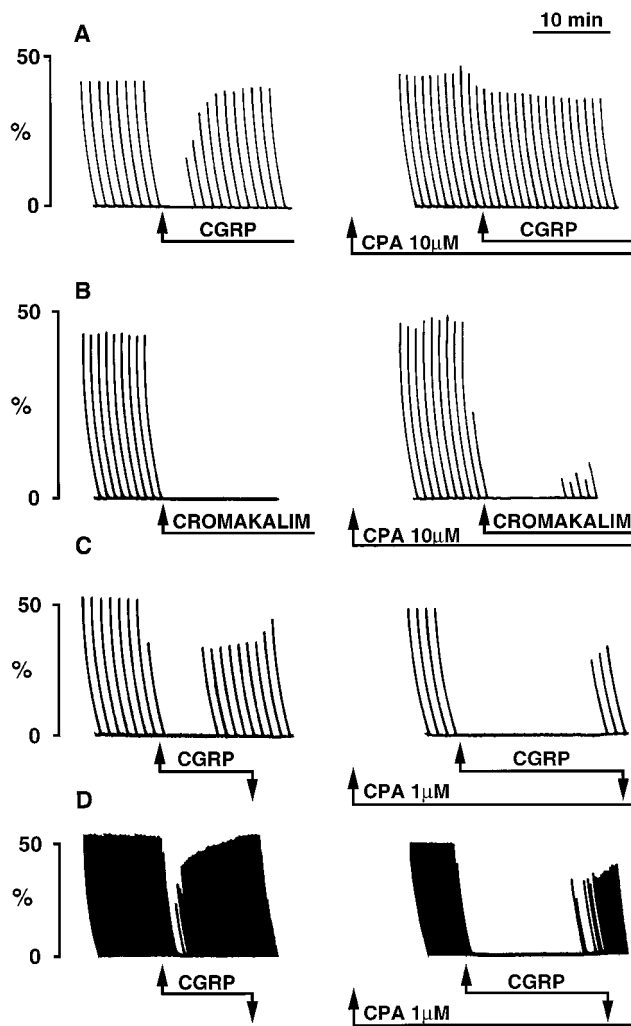
FIG. 14. Experiments illustrating the dual effect of the sarcoplasmic reticulum Ca pump inhibitor, cyclopiazonic acid (CPA) on the suppressant effect of CGRP on twitch contractions elicited by electrical stimulation of latent pacemakers in the guinea-pig ureter. The experiments demonstrate that a high concentration of CPA, assumed to empty the sarcoplasmic reticulum Ca store, blocks the ability of CGRP to suppress twitches (Panels A and B), whereas a low concentration of the drug assumed to produce a limited but incomplete inhibition of the Ca pump actually potentiates the effect of CGRP (Panels C and D). Panels A and B: Effect of 60 min exposure to 10 μM cyclopiazonic acid (CPA) on the inhibition of EFS-induced twitch contractions of guinea-pig ureter induced by 0.1 μM CGRP (Panel A) or 3 μM cromakalim (Panel C). Vertical lines are % of the maximal contraction to 80 mM KCl. Note that CGRP and cromakalim both suppress of twitches: the suppressant action of CGRP was prevented by CPA whereas the suppressant effect of cromakalim was unchanged. Panels C and D: Effect of a low concentration of cyclopiazonic acid (CPA, 1 μM for 10 min) on the inhibitory effect produced by CGRP (0.1 μM) on EFS-induced twitches of guinea-pig ureter. Electrical stimuli were delivered at a driving frequency of 1 stimulus every 60 sec (Panel C) or 15 sec (Panel D): CPA markedly prolonged the suppressant effect of CGRP at both driving frequencies. Note that when ureters were driven at a higher frequency (Panel D) the duration of the suppressant effect of CGRP is shorter than at a lower driving frequency (Panel C). Reprinted with permission from Maggi et al. (1996a).

channels in the action of CGRP can be excluded because charybdotoxin and apamin failed to affect the CGRP-induced hyperpolarization of guinea pig ureter smooth muscle.

On the basis of the above, we speculated that CGRP, by releasing Ca from the sarcoplasmic reticulum (via

cAMP and PKA), produces a transient elevation of intracellular Ca in the vicinity of the plasma membrane to activate a class of glibenclamide-sensitive K channels. To verify whether a similar mechanism could be at work in the smooth muscle of the guinea pig ureter, we studied the effect of caffeine, which releases Ca from the internal store. We found that, similar to CGRP, caffeine causes a transient, glibenclamide-sensitive hyperpolarization of the smooth muscle of the guinea pig ureter (Maggi et al., 1996c; fig. 7).

Together, these results raise the question as to whether glibenclamide-sensitive K channels in the guinea pig ureter might be Ca dependent. Although ATP-sensitive K channels are usually considered to be Ca independent, their heterogeneity (Ashcroft and Ashcroft, 1990; Beech, 1997 for reviews) leaves open the possibility that certain classes are modulated by an elevation of [Ca]$_i$. Indeed, certain ATP-sensitive K channels are blocked by an elevation of [Ca]$_i$ (e.g., Kakei and Noma, 1984; Findlay, 1987; Hussain and Wareham, 1994; Hehl et al., 1994): thus it may be speculated that in the presence of cyclopiazonic acid, ryanodine, or thapsigargin the persistent elevation of free [Ca]$_i$ inactivated ATP-sensitive K channels, thus blocking the glibenclamide-sensitive action of CGRP. However, this interpretation does not account for the glibenclamide-sensitive hyperpolarization produced by caffeine.

However, ATP-sensitive K channels are activated by an elevation of [Ca]$_i$ in the rat portal vein (Kajioka et al., 1990). Moreover, a reduced hyperpolarization response to cromakalim has been noted in Ca-free medium (e.g., Nakao et al., 1988; Gelband et al., 1989). That the K channel activated by CGRP and cromakalim in the guinea pig ureter may be Ca-sensitive is indirectly supported by the observation that superfusion with a Ca-free medium reduced the hyperpolarization induced by either agent (Maggi et al., 1996a).

According to the model proposed (fig. 13B), it was speculated that a mild impairment of Ca reuptake by the sarcoplasmic reticulum, at a time when the internal store had not yet been depleted by the test drug, should actually *prolong* the amplitude/duration of the Ca transient induced by CGRP and this effect should, in turn, result in a potentiation of twitch suppression by the neuropeptide (Maggi et al., 1996a). To address this point we studied the effect of a short time exposure (10 min) to a low concentration (1 μM) of cyclopiazonic acid and found that this treatment markedly potentiates the twitch suppression induced by CGRP, an effect opposite to that produced by a prolonged exposure (60 min) to a high concentration (10 μM) of the drug (fig. 14). According to this result, the transient nature of twitch suppression by CGRP and especially the duration of the twitch-suppressant effect would be markedly influenced by the degree of efficiency of the sarcoplasmic reticulum Ca pump in removing Ca released by CGRP.

4. Influence of "exercise" and glucose metabolism on the action of calcitonin gene-related peptide. We had noted previously (Maggi *et al.*, 1987b) that the duration of the glibenclamide-sensitive twitches-suppressant effect of CGRP in the ureter is both transient and inversely related to the frequency of evoked ureteral contractions. Therefore, it appeared of interest to investigate this aspect of CGRP action in relation to the proposed chain of events underlying its mechanism of action at cellular level (fig. 13B). Glibenclamide-sensitive K_{ATP} channels are thought to provide a link between cell metabolism and excitability (see Ashcroft and Ashcroft, 1990 for review).

We therefore studied the effect of "exercise," produced by increasing the frequency of EFS driving, on the response to CGRP. Ureteral twitches were evoked by EFS in the absence and presence of glibenclamide at different driving frequencies: in these conditions, we found that the intensity/duration of the K channel opener action of CGRP is dramatically reduced by "exercise" (fig. 15), although the action of cromakalim is unchanged (Maggi *et al.*, 1996c). In addition, metabolic impairment, induced by replacing 80% of glucose with 2-deoxyglucose, markedly potentiated the action of CGRP and counteracted the adverse effect of "exercise" on K channel activation by CGRP (Maggi *et al.*, 1996c). Notably, "exercise" did not reduce the inhibitory effect produced by CGRP in the presence of glibenclamide (Maggi *et al.*, 1996c).

Using sucrose gap, we found that the peak amplitude and duration of membrane hyperpolarization induced by CGRP were markedly diminished by "exercise," whereas the hyperpolarization induced by cromakalim was unaffected (Maggi *et al.*, 1996c; fig. 16). A short period of application (10 min) of a low concentration (1 μM) of cyclopiazonic acid, assumed to produce a limited impairment of reuptake properties of the sarcoplasmic reticulum Ca pump but not a depletion of the Ca store, fully counteracted the adverse effect of "exercise" on the twitch-suppressant effect of CGRP (Maggi *et al.*, 1996a). Altogether, these findings demonstrate that, during an increase in the working load to the smooth muscle, the ability of CGRP to activate K channels in the ureter is decreased, although K channels themselves are still readily available for activation by cromakalim.

The effect of "exercise" may be explained by an acceleration of metabolism, involving changes in ATP levels. It is known that smooth muscles have a very low or absent reserve of high energy phosphates, which are produced at demand in relation to changes in energy requirements (e.g., Paul, 1983; Ashoori *et al.*, 1984). Accordingly, a close relationship exists between the contraction-relaxation cycle and carbohydrate metabolism in smooth muscles (see Lundholm *et al.*, 1977, for review). As an example, cAMP elevation induced by β-adrenoceptor stimulation in intestinal smooth muscle is accompanied by a fast, transient, and profound (approximately 50%) reduction of ATP levels, followed by a more
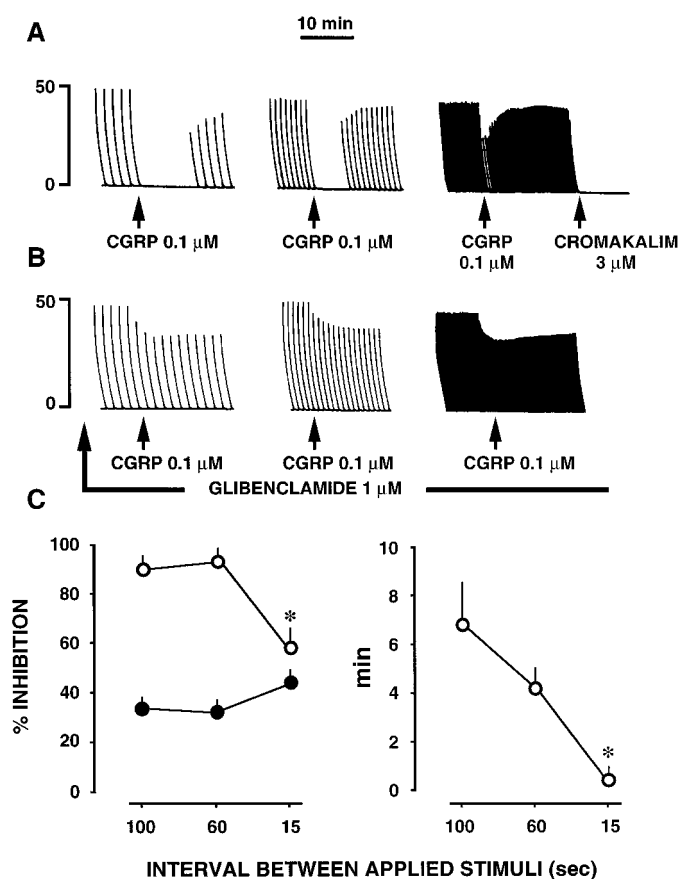

FIG. 15. Tracings (panels A and B) and quantitative data (panel C) showing the inverse relationship existing between the frequency at which guinea-pig ureters were driven by EFS and intensity/duration of the inhibitory action of CGRP in the absence and presence of 1 μM glibenclamide. In panels A and B vertical bars are % of the response to KCl. The ureters were driven by EFS by applying one stimulus every 100, 60 or 15 sec for 30 min before application of CGRP: in panel A note that the suppressant action of CGRP is almost abolished after a 30 min period of driving with stimuli applied every 15 sec, whereas the suppressant effect of cromakalim is preserved. Panel C, left side, shows the intensity of peak inhibitory action of CGRP in relation to driving frequency in the absence (empty circles) and presence (filled circles) of 1 μM glibenclamide. Panel C, right side shows the inverse relationship ($r = 0.997$, $n = 3$, $P < 0.01$) between EFS driving frequency and duration (in min) of the suppressant effect of CGRP. In lower panels, each values is mean $\pm$ s.e.m. of 5–10 experiments. * significantly different from values obtained with 60 sec interval between the applied stimuli, $P < 0.05$. Reprinted with permission from Maggi *et al.* (1996c).

prolonged increase (Andersson, 1972; Andersson and Mohme-Lundholm, 1970). Thus it may be postulated that exercise is accompanied by a faster rate of ATP synthesis in ureteral smooth muscle, rendering K_{ATP} channels less prone to activation by CGRP. However, "exercise" can also stimulate the Ca ATPase that regulates Ca handling from sarcoplasmic reticulum, thereby reducing the effect of Ca mobilization on K channel activation.

In summary, the pharmacological analysis of CGRP action in the smooth muscle of the ureter has indicated the existence of two distinct mechanisms producing smooth muscle relaxation that can be separated by the use of the K_{ATP} channel blocker, glibenclamide. One mechanism, which is glibenclamide-sensitive, involves
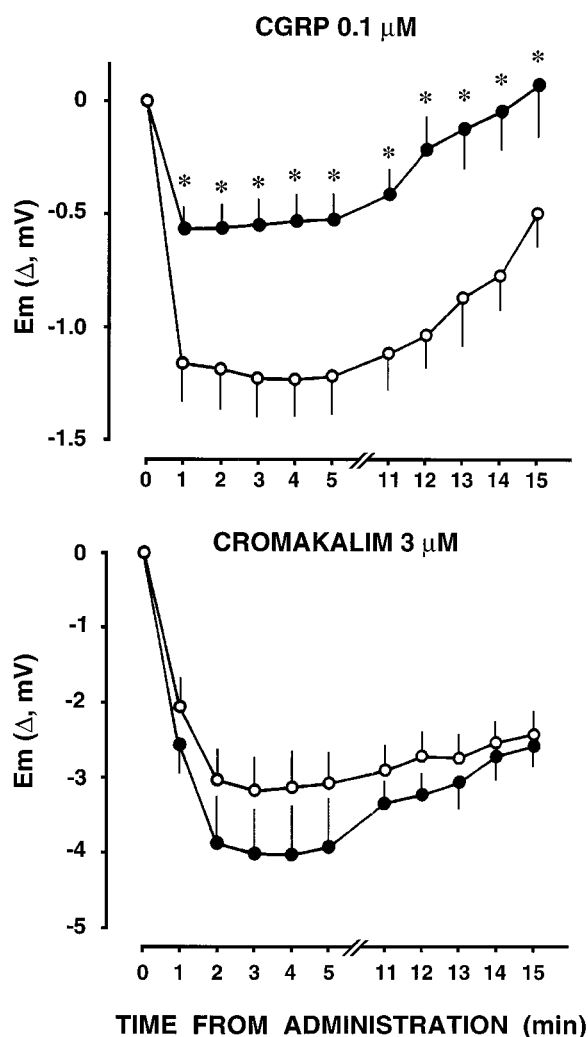
CGRP 0.1 μM

CROMAKALIM 3 μM

TIME FROM ADMINISTRATION (min)

FIG. 16. Time course of membrane hyperpolarization produced by 15 min superfusion with CGRP (0.1 μM) or cromakalim (3 μM) in resting conditions (empty circles) or within 3 min from the end of a 30 min conditioning period of " exercise" (filled circles) produced by applying electrical stimuli every 15 sec. Vertical axis shows changes (D in mV) in membrane potential (Em) recorded at various times after start of superfusion with CGRP or cromakalim. Each value is mean $\pm$ s.e.m. of 6 experiments. * significantly different from controls, $P < 0.05$. Reprinted with permission from Maggi et al. (1996c).

membrane hyperpolarization and is capable of exerting a profound suppressant effect on phasic contractions of the ureter (and ureteral peristalsis), by preventing the firing of action potentials sustained by the excitation of by latent pacemakers. This effect is causally related to the initial elevation of cAMP levels induced by CGRP receptor occupancy but there is not a direct relationship between the accumulation of cAMP and activation of K channels. Similar to findings in other smooth muscles, cAMP elevation by CGRP results in PKA activation (Quayle et al., 1994; Zhang et al., 1994a,b) and, by releasing Ca from internal stores, triggers K channel activation (fig. 13B). The nature of the K channels activated by CGRP in the ureter remains to be exactly determined: on the basis of their pharmacological properties (blockade by low concentrations of glibenclamide

and barium ions, and resistance to charybdotoxin and apamin), these channels could represent a subtype of Ca-sensitive K_{ATP} channels but this point remains to be firmly assessed. An inverse relationship is apparent between metabolic stimulation (exercise) and the ability of CGRP to activate K channels in the ureter: this factor may involve a stimulatory influence of "exercise" on the activity of the sarcoplasmic reticulum Ca pump, thereby reducing the extent and duration of the Ca transient evoked by CGRP and of the resulting hyperpolarization. Exhaustion of the CGRP-sensitive Ca store after CGRP challenge may account for the transient nature of CGRP-induced hyperpolarization despite the progressively increasing elevation of cAMP. Moreover, because K_{ATP} channel function is potently regulated by metabolic factors, such as changes in the levels of ATP and ADP (Ashcroft and Ashcroft, 1990; Beech, 1997), the possibility cannot be ruled out that CGRP itself may act as kind of "metabolic stressor" in activating K_{ATP} channels.

The nature of the second component of CGRP-induced relaxation in the ureter is less defined presently. This mechanism of relaxation appears to be more effective for inhibiting tonic type contractions of the ureter smooth muscle than the phasic contractions sustained by action potentials. The inhibitory effect of CGRP on twitches in the presence of glibenclamide develops slowly and attains a steady-state (30 to 35% inhibition of twitch amplitude) at a time when the effect of CGRP on cAMP accumulation also approaches a maximum. From a pharmacological point view, the glibenclamide-resistant component of CGRP inhibition is *not* affected by ryanodine, cyclopiazonic acid, thapsigargin, PKA inhibitors, or Rp-cAMPS. However, a delayed glibenclamide-resistant partial inhibition of twitch amplitude was also observed in response to forskolin and IBMX. Therefore, the hypothesis could be advanced that the glibenclamide-resistant component of the action of CGRP may involve cAMP accumulation and that smooth muscle relaxation is exerted via intracellular actions of this second-messenger that do not involve phosphorylation of target proteins via PKA.

D. Sensory Nerves and Inflammation

The "efferent" or "local effector" function exerted by capsaicin-sensitive afferent nerves involves the release of mediators from the peripheral endings of these primary sensory neurons (Szolcsanyi, 1984; Maggi and Meli, 1988; Maggi, 1995; Holzer, 1988). In addition to changes in smooth muscle tone, locally released sensory neuropeptides exert other effects such as vasodilatation, increase in microvascular permeability, mast cell degranulation, and recruitment of inflammatory cells. These effects are known as "neurogenic inflammation," a term that stresses the contribution of the nervous system to the initiation and maintenance of the inflammatory process. When considered as the earliest type of

response that sensory nerves set into action when interacting with potentially harmful environmental stimuli, "neurogenic inflammation" can be considered a "physiological" defense response, finalized to facilitate the removal of the *noxae* (Maggi, 1995).

Plasma protein extravasation induced by sensory nerves has been investigated in the pyeloureteral tract level. Saria *et al.* (1983) demonstrated that electrical stimulation of the inferior mesenteric ganglion caused Evans blue extravasation in the rostral third of the guinea pig ureter, whereas electrical stimulation of the pelvic nerves caused Evans blue extravasation in the caudal third of the ureters. All these responses were absent in capsaicin-pretreated animals which indicates the absolute requirement of the integrity of sensory nerves. Because Evans blue binds to plasma proteins, the increased tissue content of the dye observed after nerve stimulation is thought to reflect the extravascular accumulation of plasma proteins. This is an accepted quantitative index of the intensity of tissue edema/inflammation induced by the test stimulus. By using this technique, it was found that exogenously administered tachykinins, SP and NKA, produce plasma protein extravasation in the rat ureter (Maggi *et al.*, 1987a). Abelli *et al.* (1989) compared the activity of different tachykinin receptor selective agonists and presented evidence indicating that NK_1 receptors produce plasma protein extravasation in the ureter. Santicioli *et al.* (1993) showed that plasma protein extravasation induced by SP or a selective NK_1 receptor agonist are blocked by a selective nonpeptide tachykinin NK_1 receptor antagonist and that this effect of tachykinins is independent from the generation of NO. The involvement of tachykinin NK_1 receptors in increasing microvascular permeability to plasma protein is consistent with the analysis of their distribution in venules and arterioles of the ureter (Sann *et al.*, 1992). Finally, Nagahisa *et al.* (1992) showed that an NK_1 receptor antagonist blocks capsaicin-induced plasma protein extravasation in the guinea pig ureter.

Summarizing, the activation of the "efferent" function of sensory nerves is capable of producing a local inflammatory response in the ureter. It may be speculated that neurogenic inflammation occurs in the pyeloureteral tract during events that are of pathophysiological relevance for this organ. As an example, a damage of the urothelium produced by the passage of a stone can induce a backflow of urine in the ureteral wall and, by activating afferent nerves, can induce a local inflammatory response. In a similar way infections of the pyeloureteral tract, through the action of products of bacterial metabolism or indirectly, by altering urothelial permeability and inducing urine backflow, can activate neurogenic inflammation.

VI. Pyeloureteral Reflexes

Afferent nerves in the renal pelvis and ureter are the trigger point for the activation of several reflex responses. Although the significance of some of these re-

flexes is not fully understood, it is evident that noxious stimuli or "energies of activation" that are potentially noxious or tissue damaging are the most effective activators of both pyeloureteral reflexes and pain. In addition, certain reflex responses can be activated by mechanical stimuli and/or changes in the chemical composition of the urine and may be important for regulating urine production, water, and Na balance (Stella and Zanchetti, 1991 for review).

A. Afferent Nerves and Reflexes Arising from the Renal Pelvis

Both mechano- and chemoreceptors are present in the renal pelvis (Stella and Zanchetti, 1991). Mechanoreceptors in the renal pelvis are sensitive to changes in urine outflow pressure (Beacham and Kunze, 1969; Kopp *et al.*, 1984). Genovesi *et al.* (1993) found that an increase in urine flow rate and the corresponding increases in renal pelvic pressure closely match the increase in afferent renal nerve activity, whereas the changes in renal blood flow or perfusion pressure do not correlate with changes in renal afferent nerve discharge. Genovesi *et al.* (1993) concluded that changes in water excretion, via changes in renal pelvis pressure, are a primary factor for regulating renal afferent nerve activity.

Chemoreceptive afferent units in the renal pelvis have been extensively investigated in rats and have been classified as R1 and R2 units (Recordati *et al.*, 1978, 1980a,b, 1981). R1 units are unresponsive to mechanical stimuli, are silent under normal conditions, are activated during ischemia and may be located around blood vessels. R2 units exhibit a resting discharge and are sensitive to backflow of concentrated urine (nondiuretic urine). R2 units are sensitive to the chemical composition of the urine, can sense changes in the concentration of Na and K, and are also activated by chemicals normally present in the urine, such as bradykinin. Finally, R2 units (but not R1 units) are sensitive to capsaicin (Szolcsanyi, 1984).

Katholi *et al.* (1983) also showed that chemoreceptors in the renal pelvis but not in the ureter are activated by perfusing adenosine in the renal pelvis. The relationships existing between the activation of different populations of afferent nerves in the renal pelvis and the activation of specific reflexes is still a matter of investigation. However, R2 chemoreceptors contribute the greatest part of afferent nerve discharge to increased pressure in the renal pelvis and, therefore, work as both chemo- and mechanoreceptors (Moss and Karastoianova, 1997).

It seems that R2 chemoreceptors are implicated in sensing the concentrations of chemicals present in the renal medullary interstitial fluid and in the final urine and that their tonic activity is reduced under conditions of diuresis. Rogenes (1982) speculated that the renorenal reflex initiated by the stimulation of R2 chemore-

ceptors represents a positive feedback to maintain a low rate of water and Na excretion during antidiuresis.

The studies from Kopp and coworkers provide a stimulating link between mechanical stimuli applied to the renal pelvis, the efferent function of sensory nerves (local release of sensory neuropeptides) the activation of afferent nerves and the reflex modulation of kidney function. Kopp and Smith (1991) first observed that the administration of SP into the rat renal pelvis increases the afferent discharge of renal nerves and induces a contralateral reflex diuretic and natriuretic response mimicking that produced by increasing ureteral pressure. This reno-renal reflex is abolished by pretreatment with neurotoxic doses of capsaicin (Kopp and Smith, 1993a). Moreover, the reno-renal reflex evoked by mechanoceptor stimulation, as well as that induced by infusion of SP, are selectively reduced by the intraureteral administration of the tachykinin NK_1 receptor antagonist CP 96345 (Kopp and Smith, 1993a). Gontijo and Kopp (1994) also showed that CGRP, when administered into the renal pelvis, determines a concentration-dependent increase in the contralateral afferent nerve discharge, although the perfusion with a CGRP receptor antagonist did not affect the response to mechanoreceptor stimulation. More recently, the same group reported that: (a) both bradykinin and prostaglandins activate the same reflex that is activated by mechanical stimulation of the renal pelvis; (b) endogenous prostanoids amplify the afferent discharge induced by capsaicin, SP, bradykinin, and increased ureteral pressure; and, (c) increased ureteral pressure induces an indomethacin-sensitive release of SP in the rat renal pelvis, which suggests that the mechanically evoked reno-renal reflex requires the release of SP and activation of NK_1 receptors, via generation of prostanoids (Kopp and Smith, 1993b; Kopp et al., 1996, 1997).

Therefore, an inhibitory reno-renal reflex (i.e., stimulation of afferent reduces the sympathetic outflow to the kidney, resulting in diuresis and natriuresis) can be activated by noxious stimulation applied to the renal pelvis: this response can be activated during pathological conditions that increase the intraureteral pressure such as pyelonephritis or presence of renal stones, and when normal constituents of the urine (KCl, hyperosmolarity, low pH, or bradykinin) or products of bacterial metabolism penetrate through the urothelium to stimulate sensory nerves. At the renal pelvis level, a generation of prostanoids and a local release of sensory neuropeptides could both be involved in initiating this response. Recently Kopp et al. (1998) reported that the natriuretic reno-renal reflex initiated by distension of the renal pelvis is impaired in spontaneously hypertensive rats (SHR) and suggested that this deficit could contribute to the Na retention observed in these animals. When addressing the mechanisms of this deficit, they observed that the increase in renal afferent activity produced by administration of phorbol esters, to activate

protein kinase C, was equally evident in SHR and control rats. Moreover, the release of prostaglandins provoked by distension of the renal pelvis was also evident in both SHR and controls rats. In contrast, distension of the renal pelvis evoked a smaller release of SP in SHR as compared with control rats. Finally, exogenous SP failed to increase renal afferent activity in SHR animals (Kopp et al., 1998). The observed changes suggest that an alteration in the sensory SP innervation at both pre- and postjunctional level may account for the impairment of the reno-renal reflex observed in SHR animals.

B. Afferent Nerves and Reflexes Arising from the Ureter

Cervero and Sann (1989) presented a detailed analysis of the stimuli capable of exciting afferent nerves in the guinea pig ureter. They found that the majority of afferent nerves at this level have conduction velocities in the C-fiber range. On the basis of their mechanosensitivity, afferent nerves in the guinea pig ureter have been divided in two main groups: (a) U1 units (9% of all mechanosensitive units) that respond to contractions of the ureter and do not show ongoing activity or afterdischarge to mechanical stimulation; U1 units have a low threshold to intraluminal distension and respond after a short latency to mechanical stimuli; and (b) U2 units (91% of all mechanosensitive units) that do not respond to contractions of the ureter, have low frequency spontaneous activity (< 2.5 Hz) and exhibit a prolonged afterdischarge to mechanical stimuli; U2 units respond after a long latency (> 3 sec) to increases of intraluminal pressure in the range of 5 to 30 mmHg (Cervero and Sann, 1989). The movement of an intraluminal glass bead under the receptive field evoked strong responses of U2 units. Cervero and Sann (1989) speculated that U1 units are tension receptors placed in series with smooth muscle and monitor the normal peristalsis of the ureter, whereas U2 units are likely involved in signaling noxious events. U2 units are not excited by active contraction although they can be excited by compression or distension; U2 units also were reported to be chemosensitive, being excited by K, bradykinin, and capsaicin, much like the polymodal nociceptors commonly observed in other viscera.

Sann (1998) recently readdressed the issue of chemosensitivity of U1 and U2 units in the guinea pig ureter. He found that various chemical stimuli such as urine ($>$ 800 mosm/liter), bradykinin, or capsaicin are capable of stimulating both U1 and U2 units: however, the evoked excitation of U1 units closely follows the phasic contractions induced by applied chemical stimuli whereas the induced excitation of U2 units does not show a close relationship with induced contractions. Sann (1998) reported that an elevation of extracellular K mimics the excitatory effect of urine, whereas the application of hyperosmolar solutions or the application of Na or urea did not match the excitatory effect produced by urine.

Moreover, U2 units displayed sensitization to applied mechanical stimuli after chemical stimulation.

Afferent units with characteristics of U1 and U2 units as described in guinea pig ureter also were detected in the chicken ureter. In this species, a third population of afferent nerves also has been detected (U-G units) which are thought to code electrical activity arising from intramural ganglion cells which project to adjoining viscera (colo-rectum, cloaca) (Hammer et al., 1993).

Relatively little information is available concerning the nature of reflex responses initiated by stimulation of ureteral afferents. Amann et al. (1988b) showed that repetitive electrical stimulation of ureteric nerves evoked a slow excitatory postsynaptic potential (sEPSP) in neurons of the guinea pig inferior mesenteric ganglion (IMG); the sEPSP was abolished by pretreatment with capsaicin that itself depolarized IMG ganglion cells. Distension of the ureter or intraureteric application of capsaicin also produced a depolarization of capsaicin-sensitive IMG neurons (Amann et al., 1988b). Superfusion of the IMG with exogenous tachykinins depolarized ganglion cells and partly blunted the ganglionic sEPSP to electrical stimulation, suggesting a mediator role of these neuropeptides (Amann et al., 1988b,c). Intense (putatively noxious) stimulation of afferent nerves also induces transient changes in cardiovascular function that are interpreted as pseudoaffective equivalents of visceral pain (see Section VII.).

VII. Ureteral Pain

Pain is the only conscious sensation evoked by stimulation of the ureter in humans: pinching, cutting, heating, cooling of the outer surface of the renal parenchyma do not elicit any sensation whereas distension of the kidney, pelvis, or ureter or electrical stimulation of the renal pelvis or ureter results in intense pain (see McLellan and Goodell, 1942; Ammons, 1989; Cervero, 1994 for review)

Studies in vitro (Cervero and Sann, 1989; Hammer et al., 1993; Sann, 1998) have shown that the ureter is innervated by a large population of afferents with high activation thresholds (mean = 34 mmHg in guinea pigs) that do not respond to peristalsis and show long afterdischarges to mechanical stimuli (U2 units). U2 units are obvious candidates for transmitting the information producing pain because the pain threshold in humans is reached at a mean ureteral pressure of approximately 30 mmHg (Risholm, 1954) and pseudoaffective responses are evoked in animals at ureteral pressures in the range of 25 to 30 mmHg (Beacham and Kunze, 1969; Roza and Laird, 1995; Sann, 1998). This response is inhibited by drugs that are effective in relieving pain of renal colic (Laird and Cervero, 1996). As an example, Sann (1998) showed that increases of intraluminal pressure or application of capsaicin to the guinea pig ureter caused a transient increase in systemic blood pressure, heart rate, and changes in respiratory frequency, all indicative

pseudoaffective pain responses. A smaller population of units with a low threshold for activation (mean = 8 mmHg) that respond to peristaltic contractions (U1 units) also were found that presumably monitor peristalsis (Cervero and Sann, 1989; Hammer et al., 1993). The latter may be involved in regulating reflexes (see above) but also can encode stimuli in the noxious range and may contribute to the genesis of ureteral pain.

By checking the induction of changes in systemic blood pressure (pseudoaffective response) as an index of effective painful stimulation, Matsumoto et al. (1996) used the fos-labeling technique to identify neurons in the rat spinal cord that receive noxious afferent input from the ureter. They found that after unilateral stimulation of the ureter, fos^+ cells are present bilaterally at L1-L2 and L6-S1 spinal levels: fos^+ cells are found in the superficial medial and lateral dorsal horn and in the sacral parasympathetic nucleus. More recently, Avelino et al. (1997) developed a novel model of acute ureteral occlusion in the rat to study fos expression in the dorsal spinal cord. They found that fos expression peaks at L1-L2 and involves neurons in laminae I, IV-V, VII, and X. Labeling in lamina I was mainly ipsilateral and was ascribed to a nociceptive input giving rise to conscious pain. Labeling in the deeper laminae was observed both ipsi- and contralaterally and was suggested to be associated with the activation of autonomic reflexes. Capsaicin pretreatment abolished the induced fos labeling at all levels of the spinal cord.

A few studies have investigated the responses of spinal dorsal horn neurons to noxious stimulation of the ureter: Laird et al. (1996) found that approximately 42% of rat dorsal horn neurons in T12-L1 spinal segments with an ipsilateral somatic receptive field are also excited by noxious distension of the ureter (80 mmHg for 30 sec) in rats. For intraluminal pressure values > 20 mmHg, these neurons showed an increased firing with long latencies and afterdischarges. Moreover, all neurons with ureter afferent input also had a somatic nociceptive input: stimulation of the ureter produces changes in the somatic receptive field area, indicating a degree of plasticity in the ureteric nociceptive pathway (Laird et al., 1996).

Animal models of experimental ureteral calculi have been developed. The complete obstruction of the ureter provoked restlessness and other behavioral alterations in conscious sheep (Moriel et al., 1990), but did not evoke referred hyperalgesia or overt signs of spontaneous pain behavior. Electrical stimulation of the ureter induces pain and muscular hyperalgesia in rats. Giamberardino et al. (1988) reported that vocalization electric thresholds in muscles of the lower back were significantly reduced after ipsilateral painful electrical stimulation of the ureter. Moreover, the implantation of an experimental calculus in rats results in referred hyperalgesia and "crises" of abdominal stretching, indicative of spontaneous visceral pain (Giamberardino et al., 1990; 1995a).

Interestingly, a ligature of the ureter did not produce hyperalgesia on its own, indicating that ureteral occlusion as such is not the cause of pain. The combined administration of an antimuscarinic agent and of a COX inhibitor completely eliminated the referred hyperalgesia induced by artificial calculi in rats (Giamberardino *et al.*, 1995b). Laird *et al.* (1997) showed that partial obstruction induced by placement of an artificial calculus in the upper ureter produces a marked increase in the amplitude of ureteral contractions along with a slight reduction in their frequency and a decrease in the baseline pressure. However, total obstruction or ligature of the ureter totally abolished ureteral motility. The results suggested that increased motility caused by a stone likely contributes to the development and maintenance of visceral pain and of the referred hyperalgesia that persists even after elimination of the stone. The increased amplitude of ureteral contractions is thought to reflect an amplifying effect of stretch: this could involve, in principle, a direct effect on smooth muscle, the production of prostanoids, the release of tachykinins from afferent nerves or the passage of urine into the ureteral wall because of a damage of the mucosa (Laird *et al.*, 1997). However, the decrease in baseline pressure observed below the obstruction could involve the smooth muscle relaxant action of CGRP (Laird *et al.*, 1997).

Giamberardino *et al.* (1996) recorded changes in cell activity in the ipsilateral spinal cord (T11-T12) in rats with hyperalgesia of the *obliquus externus* muscle induced from artificial calculi of the ipsilateral upper ureter. They found a significantly higher number of spontaneously active cells indicating central sensitization in this model of referred hyperalgesia from ureteral calculi. Changes in the characteristics of spinal neurons receiving ureter afferent input after implantation of an experimental ureteral stone were also reported by Roza *et al.* (1998): these investigators observed that the presence of an ureteral stone increases the background activity and the number of ureter-driven cells in the dorsal horn of the rat spinal cord. Moreover, they reported that in rats with experimental calculi there are neurons which respond to both ureter distension and innocuous somatic stimuli, which are not usually found in normal rats: Roza *et al.* (1998) speculated that this kind of change may account for referred hyperalgesia induced inpatients with a ureteral stone by innocuous somatic stimuli.

On the background of the existence of a prominent capsaicin-sensitive innervation of the renal pelvis and ureter, Bultitude (1995) attempted the intraureteric instillation of capsaicin in humans, aiming to produce pain relief in patients suffering from loin pain/hematuria syndrome. A preliminary study in a small number of patients resulted in a lasting ($>$ 2 months) relief of pain symptoms without evidence for adverse effects on renal function (Bultitude, 1995). In a subsequent study, saline distension of the renal pelvis was used as a standard painful stimulus to provide an objective measurement of the effect of intraureteric capsaicin administration (Allan *et al.*, 1997). In that study, capsaicin pretreatment was found to produce a significant and lasting effect on distension-induced pain.

VIII. Pathophysiological Significance of the Sensory Innervation of the Pyeloureteral Complex

As evidenced in this review, the capsaicin-sensitive sensory innervation of the pyeloureteral complex can activate several local and reflex responses which affect the production of urine and modify ureteral peristalsis. As discussed in Section VI, it appears that the threshold energy of mechanical stimuli for activating capsaicin-sensitive sensory nerves largely exceeds that produced during normal ureteral peristalsis. Various chemical agents that are adequate stimuli for exciting these afferent nerves are not normally present in their interstitium but, being present into the urine, could come in contact with capsaicin-sensitive afferent nerve endings when the urothelium were damaged or when its permeability characteristics were altered.

When released by appropriate stimuli, tachykinins and CGRP profoundly affect the excitability and contractility of pyeloureteral smooth muscle by stimulating specific receptors: the differential expression of receptors/coupling mechanisms determines specialized motor patterns which are suited to facilitate the expulsion/removal of potentially harmful substances present in the urine (Maggi *et al.*, 1988b; 1992b).

The prevailing response observed upon stimulation of sensory nerves in the renal pelvis is a reinforcement of the spontaneous chrono- and inotropic activity driven by natural pacemakers: this could increase the frequency of ureteral peristalsis and facilitate urine transport to the bladder by increasing the coupling of oscillators in the renal pelvis and the efficiency of impulse transmission at the pyeloureteral junction. On a whole, this effect could facilitate the orthodromic transport/elimination of irritants coming in contact with the urothelium of the renal pelvis.

Excitatory responses can be produced by tachykinins in the ureter by exciting latent pacemakers. It is clear, however, that the prevailing effect produced by stimulating neuropeptide release in the ureter is a suppression of latent pacemakers, an effect mediated by CGRP through its K channel opener action. Endogenous CGRP, released from sensory nerves, provides a neurogenic contribution to the refractory period of ureteral smooth muscle and, in this way, contributes to set the maximal frequency of ureteral peristalsis (Maggi and Giuliani, 1994a). Moreover, endogenous CGRP can suppress the propagation of impulses along the ureteral smooth muscle: this effect involves the activation of glibenclamide-sensitive K_{ATP} channels (Meini *et al.*, 1995). CGRP provides a neurogenic "brake" producing a local suppression

of the action potential and blocking the propagation of ortho- or antidromic peristaltic waves in the ureteral smooth muscle.

The observation that the ability of CGRP to suppress latent pacemakers in smooth muscle is inversely related to the driving frequency of stimulation has important consequences for the understanding of the role of this neuropeptide in the local regulation of peristalsis. Stimuli producing CGRP release (presence of stone, infections, breakdown of urothelial barrier and intramural penetration of chemicals present in the urine) also have the ability to concomitantly excite latent pacemakers in the smooth muscle, giving rise to antiperistaltic waves and urine backflow toward the kidney. The concomitant excitation of sensory nerves, producing a local release of CGRP, blocks this event through K channel activation. However, the local suppression of excitability by CGRP would also block the orthodromic propagation of the peristaltic wave. An increased frequency of peristalsis is the first mechanism for increasing urine transport during stimulation of diuresis (Weiss, 1992). The transient and local conduction block produced by CGRP appears to be suited to prevent antiperistalsis especially during low frequency of ureteral peristalsis, when an antiperistaltic wave would have a greater chance to propel urine back toward the kidney. The CGRP-mediated local conduction block may become less and less important when the frequency of peristalsis is high: in that case, the antiperistaltic wave of excitation would have a high chance to collide with orthodromically propagated waves of excitation. We propose that the inverse relationship existing between the frequency of ureteral contractions and the intensity/duration of CGRP-induced blockade via K channels enables CGRP to locally block the excitability of the ureter in relation to varying physiological needs of urine transport. Moreover, by producing local suppression of latent pacemakers and preventing antiperistalsis CGRPcan protect the kidney from ascending infections.

The myogenic properties of the pyeloureteral smooth muscle, as reviewed in the first part of this article, seem largely sufficient to account for normal ureteral peristalsis. However, the local efferent and afferent function of capsaicin-sensitive sensory nerves are likely to affect ureteral peristalsis under pathophysiological conditions such as those occurring during a passage of a stone or a bacterial infection.

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