Br. J. Pharmac. (1984), **83**, 935–941

Systemic capsaicin treatment impairs the micturition reflex in the rat

Ulrike Holzer-Petsche & Fred Lembeck

Department of Experimental and Clinical Pharmacology, University of Graz, A-8010 Graz, Austria

1 Filling the urinary bladder via a urethral cannula and preventing its voiding in anaesthetized rats led to rhythmic contractions of the detrusor muscle, which lasted for more than 1 h. This rhythmic activity ceased about 30 min after a s.c. injection of 50 mg kg^{-1} capsaicin. The contractile response of the detrusor to topically applied capsaicin was lost after systemic administration of the toxin, whereas no change in the sensitivity to acetylcholine was observed.

2 Urinary bladders of normal rats had a capacity of about 1 ml. Bladders of rats treated with capsaicin as neonates held a volume of more than 5 ml without contracting. Such bladders were insensitive to topically applied capsaicin but they contracted to acetylcholine as strongly as the bladders of control rats.

3 During an observation period of 3 days control rats gained weight at night and lost weight by day. Rats treated with capsaicin as neonates showed little fluctuation in body weight. Such rats hardly excreted any urine by day although at night they excreted as much as controls.

4 A water load of 5 ml 100 g^{-1} was excreted by control rats within 3 h. Rats treated with capsaicin as neonates excreted only half as much. In addition, 50% of the water load was excreted far later by capsaicin treated rats than by controls. Few changes were observed in rats treated with capsaicin as adults.

5 It is concluded that all primary afferent fibres mediating the sensation of a full bladder are capsaicin-sensitive. An additional effect of capsaicin on renal mechanisms cannot be excluded.

Introduction

The vanillylamide derivative capsaicin is now widely used as a toxin for sensory neurones. Acute administration of capsaicin excites and later desensitizes small diameter afferent fibres, whereas systemic administration to newborn rats leads to degeneration of such neurones. Some of the affected neurones contain substance P (see Nagy, 1982). Also visceral afferents, running e.g. in the vagus or splanchnic nerves, comprise a population of capsaicin-sensitive fibres (Coleridge *et al.*, 1965; Lembeck & Skofitsch, 1982; Lundberg *et al.*, 1983; Sharkey *et al.*, 1983). It has been shown that in rats treated with capsaicin as neonates the substance P content of the urinary bladder was reduced by 84% (Holzer *et al.*, 1982). Furthermore about half of the afferent fibres and practically all substance P-immunoreactive ones were lost from the bladders of rats after neonatal capsaicin treatment; in such rats the urine volume in the bladder and the wet weight of the empty bladders at autopsy were significantly greater than in controls (Sharkey *et al.*, 1983).

The aim of the present study was to investigate the role of capsaicin-sensitive neurones in bladder function *in vivo*. In particular, the effect of capsaicin on the micturition reflex was studied. This was done by examining the bladder contractions in response to bladder filling, and also spontaneous diuresis and the time course of diuresis after water loading in rats pretreated with capsaicin.

Methods

Animals

Male Sprague-Dawley rats (strain OFA-SD, Forschungsinstitut für Versuchstierzucht, Himberg, Austria) were used in all experiments. They were housed under a 12 h light-dark cycle and had free access to laboratory chow and tap water.

Capsaicin treatment

Some of the rats used had been treated with capsaicin (Sigma; 50 mg kg^{-1} subcutaneously (s.c.); 5 mg ml^{-1}

in 5% ethanol, 5% Tween 80 in saline) on the 2nd day of life. Rats treated as adults received 125 mg kg^{-1} s.c. divided into three doses: 25 and 50 mg kg^{-1} on the 1st and 50 mg kg^{-1} on the 2nd day. In this instance, a 20 mg ml^{-1} solution was used (with 20% ethanol, 10% Tween 80 in saline). In each case control rats were injected with the respective solvents. All injections were performed under ether anaesthesia. Rats treated as neonates were used at an age of 2–6 months, those treated as adults 4 or 7 days after the last dose of capsaicin. In order to test for the effectiveness of the treatment a wiping test was performed before the experiments. A drop of a 100 μg ml^{-1} capsaicin solution was instilled into one eye of the rats and the protective wiping movements were counted (Gamse *et al.*, 1981).

Micturition reflex

Rats weighing 300–400 g were anaesthetized with urethane (1.2 g kg^{-1} i.p.). The right carotid artery was cannulated for blood pressure monitoring. The abdomen was opened by a midline incision and a polyethylene catheter (i.d.: 1.4 mm, o.d.: 1.9 mm) inserted into the urinary bladder via the urethra as described by Maggi & Meli (1982). By means of a Y-shaped adapter the catheter was connected to a syringe and to a Statham pressure transducer. The whole system was filled with 0.9% w/v NaCl solution (saline) kept at 37°C. The abdomen was covered with cotton wool swabs soaked in warm saline.

After a stabilizing period of 10 min the bladder was rapidly filled with 0.5 ml saline. In the experiments designed to evaluate the total capacity of the bladder the volume was increased by 0.5 ml every 10 min up to a total volume of 2.5 ml in controls and 5 ml in rats treated with capsaicin as neonates (6 ml in one rat). Pressure changes due to isometric contractions of the detrusor muscle were recorded by a Watanabe Linearcorder. At the end of each experiment the actual volume contained in the bladder was measured. Then the bladders were excised, blotted and weighed.

In another set of experiments with untreated rats the bladders were filled with 0.5 ml of saline and observed for 10 min. If by then no rhythmic contractions of the detrusor had developed, the volume was increased in steps of 0.25 ml up to a maximum of 1 ml. Under these conditions regular contractions were observed in 78% of bladders. At the lowest filling volume at which rhythmic activity with an amplitude of more than 1 kPa occurred the bladders were left to equilibrate for 15 min. Then, 50 mg kg^{-1} capsaicin or the corresponding volume of solvent was injected s.c. The activity of the bladder was monitored for a further 60 min. Then the bladders were challenged with 400 μl topically applied ACh (acetylcholine-Cl, Sigma, 100 μg ml^{-1}) or capsaicin (150 μg ml^{-1}, 1:133 diluted from the solution used for the s.c. injection). This method (see Maggi & Meli, 1982) yielded reproducible bladder contractions to ACh without having any appreciable systemic effects. In each preparation capsaicin was applied only once in order to avoid desensitization. In 3 such experiments propranolol (propranolol-HCl, ICI, 0.3 mg kg^{-1}) was administered intravenously 10 min after capsaicin.

As it often took up to 5 min until stable rhythmic contractions of the detrusor muscle developed on filling the bladder, frequency and amplitude of contractions were evaluated between 5 and 10 min after increasing the bladder volume; in the experiments in which acute s.c. capsaicin was administered, control values were taken from the last 5 min before injection, i.e. 10–15 min after increasing the bladder volume.

Diuresis

Spontaneous diuresis To test spontaneous urine excretion, 6 solvent-treated rats and 6 rats treated with capsaicin as neonates were put into metabolism cages after having established the effectiveness of the capsaicin treatment by the wiping test (Gamse *et al.*, 1981). The weight of the rats, their water and food intake as well as urine excretion and defaecation were observed for 3 days divided into periods from 19 h 00 min–06 h 00 min (night) and from 07 h 00 min–18 h 00 min (day). Every 12th h was allowed for weighing the rats and measuring their intake and excretion. For each rat the 3 values of the night and day periods were averaged and the mean for each group calculated.

Diuresis after water loading The diuresis test was based on the method described by Dicker & Ginsburg (1950). Rats weighing 180–220 g were fasted for 18 h and deprived of water during the last hour before the experiment. The rats were made to empty their bladders by stressing them by means of abrupt handling and by manual pressure on the abdomen. Under ether anaesthesia the rats were fed warm tap water (5 ml 100 g^{-1} body weight) by stomach tube and put into metabolism cages. These experiments always started at approximately 09 h 00 min. The urine was collected in graded cylinders and the volume recorded at 15 min intervals for 3 h. At the end of the experiment the bladders were emptied again and the total amount of urine produced was expressed as % of water administered. As the rats treated with capsaicin as neonates did not completely empty their bladders, they were killed after the experiment by a blow on the neck. The volume of the residual urine was measured and included in the volume of

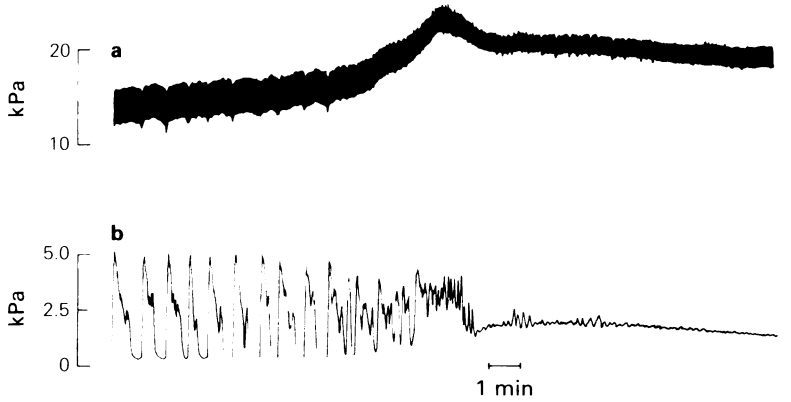

Figure 1 Recording of blood pressure (a) and bladder contractions (b) from 40 to 61 min after an injection of capsaicin 50 mg kg^{-1} s.c. Bladder contractions become irregular and cease simultaneously with a rise in blood pressure.

urine produced. In addition, the time point at which a volume of urine corresponding to 50% of the water had been excreted (i.e. expelled by the bladder) was calculated.

Statistics

Values are expressed as x ± s.e.mean. Statistical evaluation was performed using Student's *t* test.

Results

Micturition reflex

These experiments were carried out with rats that exhibited a regular contractile activity of the bladder on filling with 0.5–1 ml saline. In 6 rats injected with solvent these rhythmic bladder contractions were observed up to at least 60 min, except in one where they ceased after 54 min. The blood pressure remained stable throughout the experiments. The contractile activity was, however, abolished 33 ± 5 min ($n = 8$) after a s.c. injection of 50 mg kg^{-1} capsaicin (Figure 1, Table 1). In 6 of the 8 rats the activity became irregular before it eventually stopped. The cessation of rhythmic contractions coincided with a slow rise in blood pressure by 7.4 ± 0.9 kPa. Administration of propranolol 10 min after capsaicin ($n = 3$) did not change this pattern.

Sixty min after the s.c. injection of capsaicin or solvent both sets of rats responded equally well to the topical application of ACh (100 μg ml^{-1}). This concentration evoked contractions 75% of the maximal response to ACh. Topical application of capsaicin (150 μg ml^{-1}), which in controls elicited contractions almost as high as did ACh (100 μg ml^{-1}) (Tables 1 and 2), led to a slight contraction of 1 kPa only in one of the capsaicin-injected rats (Table 1).

The bladders of the 8 solvent-treated control rats had a capacity of 1.2 ± 0.1 ml. Every additional volume of saline injected through the urethral cannula was expelled alongside the catheter. In contrast, bladders of rats treated with capsaicin as neonates

Table 1 Effect of an injection of capsaicin (50 mg kg^{-1} s.c.) or solvent on rhythmic bladder contractions induced by filling with 0.5–1 ml saline

	Controls ($n = 6$)	*Capsaicin* ($n = 8$)
Control period:		
Amplitude (kPa)	3.0 ± 0.6	3.6 ± 0.6
Frequency (contractions min^{-1})	1.3 ± 0.2	2.1 ± 0.4
Duration of rhythmic bladder activity (min after injection)	> 59	33 ± 5**
Contraction to topical ACh (kPa)	3.2 ± 0.5	3.0 ± 0.3
Contraction to topical capsaicin (kPa)	2.0 ± 0.6	0.13 ± 0.13*

*$P < 0.01$, **$P < 0.002$, vs. controls.

Table 2 Characteristics of bladders in rats treated with capsaicin as neonates compared with controls

	Controls ($n=8$)	*Capsaicin* ($n=7$)
Bladder weight (mg)	80±3	221±24**
Total capacity (ml)	1.2±0.1	>5.0**
Maximal amplitude of bladder contraction (kPa)	3.0±0.4	0.2±0.2**
Contraction to topical ACh (kPa)	3.2±0.4 ($n=6$)	3.0±0.6
Contraction to topical capsaicin (kPa)	2.7±0.6 ($n=4$)	<0.5*

*$P<0.02$, **$P<0.001$, vs. controls.

held a volume of at least 5 ml without contracting (Table 2). Only one of 7 rats showed bladder contractions of 1.5 kPa at a volume of 5 ml. In addition, an increased wet weight of the bladders was observed in the capsaicin-treated rats (Table 2).

The bladders of capsaicin-treated rats were hardly affected by topical application of capsaicin (Table 2), whereas they were equally sensitive, as the bladders of controls to topically applied ACh.

Diuresis

Spontaneous diuresis The two groups of rats used in this experiment were of the same age and initial weight, i.e. the 6 control rats weighed 377±11 g, the 6 capsaicin-treated rats 377±9 g. The capsaicin treatment was shown to have been effective, as the treated rats wiped 5±2 times compared with a value of 19±2 in controls ($P<0.01$).

Control rats markedly gained weight during the night and lost weight during the day (Figure 2). Only minor fluctuations were observed in capsaicin-treated rats. Water and food intake were higher at night, the period of higher activity, than during the day and did not differ between capsaicin-treated and control rats. Whereas in controls the same volume of urine was excreted by day as at night, capsaicin-treated rats excreted significantly less urine than controls by day although at night there was only a small, non-significant difference (Figure 2). No differences were observed in defaecation.

Diuresis after water loading Rats treated with capsaicin as neonates or as adults 7 days before the experiment were completely desensitized to acute application of capsaicin as indicated by the wiping test (Table 3). The 7 rats which had been treated as adults 4 days before the experiment wiped 4±2

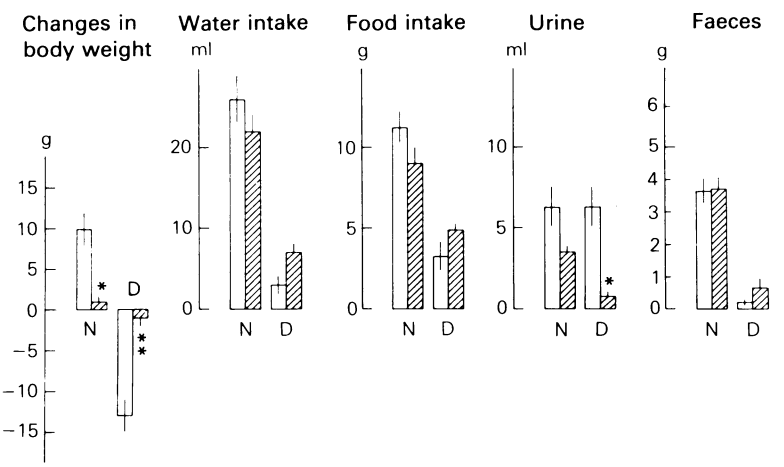

Figure 2 Metabolism of 6 solvent-treated rats (open columns) and 6 rats treated with capsaicin as neonates (hatched columns) during an observation period of 3 days. Vertical lines show s.e.mean. N: 11 hour night period; D: 11 hour day period (for details see Methods). *$P<0.01$, **$P<0.001$, vs. controls.

Table 3 Extent of desensitization and urine excretion in capsaicin-treated and control rats after water loading

	Controls ($n=14$)	*Capsaicin as neonates* ($n=5$)	*Capsaicin as adults (4 days)* ($n=7$)	*Capsaicin as adults (7 days)* ($n=6$)
Total urine produced within 3 h (% of water fed)	100 ± 2	57 ± 8***	93 ± 3*	92 ± 4
Time (min) after which 50% of the water was excreted spontaneously	83 ± 3	>170*** (>180 in 4 rats, 131 in 1 rat)	92 ± 3*	105 ± 6**
Number of wipings	23 ± 2	0 ± 0***	4 ± 2***	0.3 ± 0.2***

4 days and 7 days, number of days after treatment before experiment.
*$P<0.05$, **$P<0.002$, ***$P<0.001$ vs. controls.

times, but this value was also significantly ($P<0.001$) different from that of the controls.

Control rats (solvent-treated as neonates or as adults) excreted 50% of the water load fed by stomach tube within 83 ± 3 min ($n=14$); the volume of urine produced by 3 h corresponded to 100 ± 2% of the water fed (Table 3). In rats treated with capsaicin as neonates the volume of urine corresponded to 57 ± 8% ($n=5$) of the water intake, and only one rat had excreted 50% of the water spontaneously before the 3 h period had elapsed (at 131 min).

Only minor changes in urine excretion were observed in rats treated with capsaicin (125 mg kg^{-1} s.c.) as adults 4 or 7 days before the experiment (Table 3).

Discussion

Micturition reflex

Barrington (1931) described six reflexes as constituting the act of micturition, the most important of which is a contraction of the detrusor muscle evoked by distending the bladder. It is a polysynaptic reflex, the afferents and efferents running in the pelvic nerves, the centre lying in the pons (Kuru, 1965). The other five reflexes involve the urethra and are therefore not relevant in the present experimental setup. Increasing the bladder volume stimulates tension receptors in the bladder wall (Iggo, 1955). If voiding of the bladder is prevented, rhythmic bladder contractions develop, a discharge of parasympathetic neurones in the pelvic nerves preceding each contraction (de Groat & Ryall, 1969).

In the rat dorsal root ganglion cells labelled by injection of horseradish peroxidase into the bladder wall are small ganglion cells (Applebaum *et al.*, 1980). Therefore, most bladder afferents must have small myelinated or unmyelinated axons. Ten to 16% of the dorsal root ganglion cells labelled by True Blue injection into the bladder wall also stained for substance P immunoreactivity (Sharkey *et al.*, 1983). The target neurones of capsaicin's toxicity belong to this class of ganglion cells (Jancsó *et al.*, 1977; Nagy *et al.*, 1981). Therefore, capsaicin probably damages the first afferent neurone of the micturition reflex. The completeness of the blockade would also imply that all fibres arising from tension receptors in the bladder wall are capsaicin-sensitive.

Systemic injection of capsaicin completely abolished the rhythmic contractions constituting the efferent part of the micturition reflex after a mean time of 33 min. Capsaicin is well absorbed after s.c. administration, the peak concentration in nervous tissue being reached approximately 30 min after a s.c. injection of 50 mg kg^{-1} (Saria *et al.*, 1982). The finding that cutaneous plasma extravasation induced by local application of mustard oil was almost abolished 30 min after s.c. capsaicin injection (Lembeck & Donnerer, 1981) also corresponds with the latency of capsaicin's action observed in the present experiments.

If the rise in blood pressure observed while the bladder contractions were ceasing reflected an increased sympathetic activity, this might inhibit bladder contractions by stimulation of β-adrenoceptors (deGroat & Saum, 1972). This could, however, be excluded, as the injection of propranolol neither influenced the rise in blood pressure nor prevented the cessation of rhythmic bladder contractions. Moreover, the pressor effects of capsaicin have already been shown to be mediated by neither sympathetic nor parasympathetic mechanisms but to be caused by a direct action of capsaicin on peripheral blood vessels (Toda *et al.*, 1972; Donnerer & Lembeck, 1982).

The irregular bladder contractions preceding the complete cessation are not yet understood. They might represent the efferent response to a desynchronization of the afferent input which may be

caused by different fibres not being blocked at exactly the same time. On the other hand they might be the direct consequence of a transmitter release, probably of substance P, from the peripheral endings. This latter proposal would be in accord with the action of capsaicin on the gut (Barthó *et al.*, 1982). The released substance P is able to contract directly the detrusor muscle (Sjögren *et al.*, 1982). This mechanism may also be responsible for the bladder contractions seen after topical application of capsaicin.

It is unlikely, however, that capsaicin-sensitive neurones are involved in the efferent part of the micturition reflex or are responsible for the atropine-resistant bladder contractions, as Sharkey *et al.* (1983) demonstrated that the bladders of capsaicin-treated and control rats responded equally well to electrical field stimulation in the presence of atropine. The systemic injection of capsaicin did not affect the contractility of the detrusor muscle, as it contracted to topically applied ACh as well as in controls (Tables 1 and 2).

After neonatal capsaicin treatment of rats more than half of the bladder afferents degenerate, among which are all the substance P-containing fibres (Sharkey *et al.*, 1983). In rats so pretreated the pattern of bladder disturbance resembles the clinical syndrome of an atonic neurogenic bladder (Herbut, 1952), which can be observed in neurological diseases affecting sensory nerves (as e.g. tabes dorsalis). Cystometric investigations in patients yielded no contractions of the bladder either during filling or when the bladder was full to capacity (Herbut, 1952). After sectioning of the sacral dorsal roots in cats a prolonged overdistension of the bladder occurs due to a lack of sensation, followed by atony with great volumes of residual urine and overflow (Barrington, 1914).

Diuresis

The pattern of spontaneous urine excretion in capsaicin-treated rats corresponds with their inability to sense the fullness of the bladder. It has not been investigated what stimuli remain to elicit a contraction of the detrusor, but probably psychic stimuli as well as external pressure on the abdomen still lead to emptying of the bladder. These stimuli may occur more often during the night, the activity period of the rat, than during the day. Therefore, a sufficient emptying of the bladder may be obtained at night, but not in the daytime, when the rat is quiet and asleep most of the time. The observation that, by day, capsaicin-treated rats lose less weight than controls corresponds with the observed pattern of urine excretion, if this fact is interpreted as simple retention of urine. As, however, the intake of water and food and the weight of the faeces do not differ significantly between the two groups of rats, the difference in weight gain at night cannot satisfactorily be explained.

Fifty % of a water load was excreted far later in rats treated with capsaicin as neonates than in controls. This may be taken as an additional indication of the atony and increased capacity of their bladders. However, it was also noted that a smaller amount of urine was produced in such rats during the experimental period. This cannot be attributed to the impairment of micturition but, seems to reflect a disturbance of renal function. In the light of these findings the reduced volume of urine passed spontaneously during the day could also be caused by a decrease in urine production by the kidney.

In the guinea-pig kidney a few substance P-immunoreactive fibres were demonstrated close to tubuli (Alm *et al.*, 1978) and along blood vessels (Hökfelt *et al.*, 1978). Although in the rat similar observations have not yet been made, in this species too substance P has been found to be a very potent natriuretic and diuretic agent (Arendshorst *et al.*, 1976; Kramer *et al.*, 1983). It is tempting to speculate that capsaicin-sensitive substance P fibres in the kidney might play a physiological role in influencing renal plasma flow and tubular function.

In rats treated with capsaicin as adults the effect of the toxin on diuresis after a water load was only minor. Compared with the deficiencies occurring after neonatal capsaicin treatment, other sensory functions are less noticeably impaired and/or recover within a few days after treatment of adults, as e.g. the reaction times in the tail withdrawal and hot plate tests (Gamse, 1982). This correlates with the observation of only slight morphological changes in afferent neurones of rats treated with capsaicin as adults (Joó *et al.*, 1969), whereas after neonatal treatment up to 95% of the unmyelinated primary afferent fibres from lumbar dorsal roots degenerate (Nagy *et al.*, 1981). It, therefore, seems as if an almost complete degeneration of peptidergic small diameter afferents is necessary for disturbances in urinary excretion to become manifest.

The authors thank Mr W. Schluet for preparing the drawings and Ms I. Freiberger for typing the manuscript. The work was supported by the Austrian Scientific Research Funds and the Austrian Academy of Sciences.

References

ALM, P., ALUMETS, J., BRODIN, E., HÅKANSON, R., NILSSON, G., SJÖBERG, N.-O. & SUNDLER, F. (1978). Peptidergic (substance P) nerves in the genito-urinary tract. *Neurosci.*, **3,** 419–425.

APPLEBAUM, A.E., VANCE, W.H. & COGGESHALL, R.E. (1980). Segmental localization of sensory cells that innervate the bladder. *J. comp. Neurol.*, **192,** 203–209.

ARENDSHORST, W.J., COOK, M.A. & MILLS, I.H. (1976). Effect of substance P on proximal tubular reabsorption in the rat. *Am. J. Physiol.*, **230,** 1662–1667.

BARRINGTON, F.J.F. (1914). The nervous mechanism of micturition. *Quart. J. exp. Physiol.*, **8,** 33–71.

BARRINGTON, F.J.F. (1931). The component reflexes of micturition in the cat. *Brain*, **54,** 177–188.

BARTHÓ, L., HOLZER, P., LEMBECK, F., SZOLCSÁNYI, J. (1982). Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to release of substance P. *J. Physiol.*, **332,** 157–167.

COLERIDGE, H.M., COLERIDGE, J.C. & LUCK, J.C. (1965). Pulmonary afferent fibres of small diameter stimulated by capsaicin and by hyperinflation of the lungs. *J. Physiol.*, **179,** 248–262.

DEGROAT, W.C. & RYALL, R.W. (1969). Reflexes to sacral parasympathetic neurones concerned with micturition in the cat. *J. Physiol.*, **200,** 87–108.

DEGROAT, W.C. & SAUM, W.R. (1972). Sympathetic inhibition of the urinary bladder and of pelvic ganglionic transmission in the cat. *J. Physiol.*, **220,** 297–314.

DICKER, S.E. & GINSBURG, M. (1950). Some observations on the antidiuretic activity of rat serum. *Br. J. Pharmac.*, **5,** 497–504.

DONNERER, J. & LEMBECK, F. (1982). Analysis of the effects of intravenously injected capsaicin in the rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **320,** 54–57.

GAMSE, R. (1982). Capsaicin and nociception in the rat and mouse. Possible role of substance P. *Naunyn-Schmiedebergs Arch. Pharmac.*, **320,** 205–216.

GAMSE, R., LACKNER, D., GAMSE, G. & LEEMAN, S.E. (1981). Effect of capsaicin pretreatment on capsaicin-evoked release of immunoreactive somatostatin and substance P from primary sensory neurons. *Naunyn-Schmiedebergs Arch. Pharmac.*, **316,** 38–41.

HERBUT, P.A. (1952). *Urological Pathology*, Vol. I. Philadelphia: Lea & Febiger.

HÖKFELT, T., SCHULTZBERG, M., ELDE, R., NILSSON, G., TERENIUS, L., SAID, S. & GOLDSTEIN, M. (1978). Peptide neurons in peripheral tissues including the urinary tract: Immunohistochemical studies. *Acta Pharmac. Tox.*, **43** (II), 79–89.

HOLZER, P., BUCSICS, A. & LEMBECK, F. (1982). Distribution of capsaicin-sensitive nerve fibres containing immunoreactive substance P in cutaneous and visceral tissues of the rat. *Neurosci. Lett.*, **31,** 253–257.

IGGO, A. (1955). Tension receptors in the stomach and the urinary bladder. *J. Physiol.*, **128,** 593–607.

JANCSÓ, G., KIRÁLY, E. & JANCSÓ-GÁBOR, A. (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurons. *Nature*, **270,** 741–743.

JOÓ, F., SZOLCSÁNYI, J. & JANCSÓ-GÁBOR, A. (1969). Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin. *Life Sci.*, **8** (I), 621–626.

KRAMER, H.J., KLINGMÜLLER, D., FLACHSKAMPF, F.A. & DÜSING, R. (1983). Substance P-induced changes in kidney function in the conscious rat: Relation to the renal prostaglandin system. *Renal Physiol.*, **6,** 10–18.

KURU, M. (1965). Nervous control of micturition. *Physiol. Rev.*, **45,** 425–494.

LEMBECK, F. & DONNERER, J. (1981). Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content. *Naunyn-Schmiedebergs Arch. Pharmac.*, **316,** 240–243.

LEMBECK, F. & SKOFITSCH, G. (1982). Visceral pain reflex after pretreatment with capsaicin and morphine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **321,** 116–122.

LUNDBERG, J.M., BRODIN, E. & SARIA, A. (1983). Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. *Acta physiol. scand.*, **119,** 243–252.

MAGGI, C.A. & MELI, A. (1982). An *in vivo* procedure for estimating spasmolytic activity in the rat by measuring smooth muscle contractions to topically applied acetylcholine. *J. Pharmac. Meth.*, **8,** 39–46.

NAGY, J.I. (1982). Capsaicin: A chemical probe for sensory neuron mechanisms. In *Handbook of Psychopharmacology*, Vol 15. ed. Iversen, L.L., Iversen, S.D. & Snyder, S.H. pp. 185–235. New York/London: Plenum Publishing Corp.

NAGY, J.I., HUNT, S.P., IVERSEN, L.L. & EMSON, P.C. (1981). Biochemical and anatomical observations on the degeneration of peptide-containing primary afferent neurons after neonatal capsaicin. *Neurosci.*, **6,** 1923–1934.

SARIA, A., SKOFITSCH, G. & LEMBECK, F. (1982). Distribution of capsaicin in rat tissues after systemic administration. *J. Pharm. Pharmac.*, **34,** 273–275.

SHARKEY, K.A., WILLIAMS, R.G., SCHULTZBERG, M. & DOCKRAY, G.J. (1983). Sensory substance P-innervation of the urinary bladder: Possible site of action of capsaicin in causing urine retention in rats. *Neurosci.*, **10,** 861–868.

SJÖGREN, C., ANDERSSON, K.-E. & HUSTED, S. (1982). Contractile effects of some polypeptides on the isolated urinary bladder of guinea-pig, rabbit, and rat. *Acta Pharmac. Tox.*, **50,** 175–184.

TODA, N., USUI, H., NISHINO, N. & FUJIWARA, M. (1972). Cardiovascular effects of capsaicin in dogs and rabbits. *J. Pharmac. exp. Ther.*, **181,** 512–521.

(Received May 31, 1984.
Revised July 3, 1984.)