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Ouabain-induced increases in resting tone of human hyperplastic prostate following repeated noradrenaline and electrical field stimulation

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1 The effect of ouabain on contractions to repeated noradrenaline stimulation and electrical field stimulation of human hyperplastic prostate was examined. Ouabain (1 μM) did not induce contractile response *per se* but progressively increased the resting tone (i.e., the tone between one noradrenaline stimulation, or electrical field stimulation, and the following) of human hyperplastic prostate.

2 The increased tone by ouabain following repeated noradrenaline stimulations or electrical field stimulation was fully relaxed by the removal of external calcium, and recovered following restoration of calcium.

3 The effect of noradrenaline on Na^+ uptake was measured. Noradrenaline (10 μM) significantly increased the rate of Na^+ accumulation in the presence of ouabain (1 μM); this stimulatory effect was almost completely blocked by prazosin (0.1 μM) and ethylisopropylamiloride (100 μM). In contrast, tetrodotoxin (1 μM) had no effect on noradrenaline-stimulated Na^+ transport in human hyperplastic prostate.

4 Intracellular Na^+ loading by noradrenaline (10 μM) in the presence of ouabain (1 μM) significantly increased the transmembrane Ca^{2+} uptake as compared with the absence of ouabain; however, nifedipine (1 μM) was ineffective on Ca^{2+} uptake under this condition.

5 Transmembrane Ca^{2+} efflux was stimulated by noradrenaline (10 μM) in human hyperplastic prostate; this effect was significantly decreased in the presence of ouabain (1 μM).

6 It is suggested that the increased tone of human hyperplastic prostate following repeated excitation in the presence of ouabain is due to increased Ca^{2+} entry and reduced efflux of Ca^{2+} through the Na^+/Ca^+ exchange system as a consequence of Na^+ pump inhibition by ouabain.

Keywords: Noradrenaline; ouabain; Na^+ pump; Na^+/Ca^+ exchange; human prostate

Introduction

Benign prostatic hyperplasia (BPH), a progressive enlargement of the prostate, develops in aging men, leading to outflow obstruction and acute retention of urine. It has been suggested, on the basis of both pharmacological research (Hedlund *et al.*, 1985; Yamada *et al.*, 1987; Kitada & Kumazawa, 1987) and clinical trials (Kirby *et al.*, 1987; Jardin *et al.*, 1991), that α-adrenoceptor stimulation is an important factor in the development of urinary obstruction in BPH. Additionally, the tone of prostatic smooth muscle regulated by the autonomic nervous system is thought to be the 'dynamic' component of bladder outlet obstruction by BPH (Caine, 1986) and a dense network of adrenergic nerve fibres is found within the smooth muscle layer of human prostate (Vaalasti & Hervonen, 1980). It is suggested that endogenous adrenergic stimulation plays an important role in human prostate.

Circulating endogenous inhibitors of the plasmalemma Na^+ pump (Na^+-K^+ ATPase) could be responsible for increased vascular smooth muscle tone in some forms of hypertension (Hamlyn *et al.*, 1982; Masugi *et al.*, 1986). In addition, cardiac glycosides induce a myogenic tonic contraction in isolated vascular smooth muscle (Bova *et al.*, 1988; Woolfson *et al.*, 1990) and exert direct systemic vasoconstriction (Mason *et al.*, 1986). Furthermore, in therapeutic dosage, digoxin significantly augments pressor responsiveness to noradrenaline (Guthrie, 1984). In addition to vascular smooth muscle, the activity of Na^+-K^+ ATPase and/or Na^+ conductance is a crucial factor in contractility of nonvascular smooth muscles (Aickin *et al.*, 1984; Ohya *et al.*, 1986; Moore *et al.*, 1991).

In this context, we postulate that inhibition of the Na^+ pump in human hyperplastic prostate by endogenous factors or therapeutic cardiac glycosides administration can amplify the responsiveness to noradrenaline stimuli, and be involved in the pathophysiology of BPH. To test this hypothesis, we have studied the contractile effect of repeated noradrenaline stimulation and electrical field stimulation on the human hyperplastic prostate exposed to ouabain, and the effect of noradrenaline on Na^+ uptake as well as Ca^{2+} movement.

Methods

Human hyperplastic prostates were obtained at operation from 26 males, aged 57–79 years, by open prostatectomy or transurethral resection of the prostate. All these patients had histories of prostatism and were diagnosed to have BPH by the combination of rectal digital examinations, transrectal sonography of prostate and urodynamic studies (including uroflowmetry, urethral pressure profile and cystometry).

Isometric tension experiments

Immediately after removal, the prostatic tissues were cut into strips (3 × 15 mm) and mounted vertically in a thermostatically controlled organ bath (37°C) containing Krebs solution (5 ml) and bubbled with a mixture of CO_2 (5%) and O_2 (95%). Tis-

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sues were equilibrated for at least 90 min with four changes of solution and maintained at an optimal tension of 1 g. Contractions were recorded isometrically via a force-displacement transducer (Grass, Model 7DAG) connected to a Grass polygraph.

In electrical field stimulation experiments, prostatic strips were mounted vertically within two parallel platinum ring electrodes in organ baths containing Krebs solution (5 ml). Intramural nerve stimulation was performed by means of an electronic stimulator (Grass model S88) delivering square pulses of 0.2 ms duration at supramaximum voltage (80 V over the electrodes) and 20 Hz for 5 s. Complete inhibition of the response by tetrodotoxin (0.1 μM) confirmed that contractions induced by field stimulation were nerve-mediated.

The ouabain concentration used in this study was determined in unstimulated strips. The results showed that ouabain, at a concentration of 1 μM, exerted no contractile response within an exposure of 100 min. In all experiments, a concentration of 1 μM ouabain was then used. The prostatic strips were stimulated by noradenaline for the indicated duration and then washed out in the absence or presence of ouabain; the resting tone was recorded before each agonist stimulation.

$^{22}Na^+$ uptake measurements

For the assay of $^{22}Na^+$ uptake, human hyperplastic prostate was preincubated in Krebs solution at 37°C for 1 h. To initiate influx, 0.5 μCi ml^{-1} $^{22}Na^+$ was added to the bathing solution in the absence or presence of indicated agents for 10 min. The influx was terminated by six washes of ice-cold 100 mM $MgCl_2$. Incorporated $^{22}Na^+$ was measured in a gamma counter and corrected for protein content. Protein was determined by the method of Lowry *et al.* (1951).

$^{45}Ca^{2+}$ uptake measurements

For determination of Ca^{2+} uptake, tissues were preincubated in the absence or presence of ouabain (1 μM) for 30 min at 37°C and then noradrenaline (10 μM) was added for another 10 min. Following two washes for 20 min, tissues were incubated in 1 μCi ml^{-1} $^{45}Ca^{2+}$-containing Krebs solution for 10 min at 37°C and then washed in ice-cold Ca^{2+}-free/2 mM EGTA Krebs solution for 30 min to remove extracellular $^{45}Ca^{2+}$. The tissues were then removed, lightly blotted with No. 5 Whatman filter paper, weighed and dissolved in 37% perchloric acid at 75°C. The radioactivity was counted in a liquid scintillation counter (Packard Model 2200 CA).

$^{45}Ca^{2+}$ efflux measurements

Tissues were preincubated in $^{45}Ca^{2+}$ (1 μCi ml^{-1})-containing Krebs solution bubbled with a mixture of CO_2 (5%) and O_2 (95%) for 3 h at 37°C and for a further 30 min period in the absence or presence of ouabain (1 μM). At the time of study, tissues were washed three times in Krebs solution at 37°C and then exposed to noradrenaline (10 μM) for 5 min in the absence or presence of ouabain (1 μM). The experiment was terminated by washing the tissues four times with ice-cold, Ca^{2+}-free Krebs solution containing 10 mM $LaCl_3$, followed by a 5 min incubation with the same buffer. Determination of $^{45}Ca^{2+}$ content was performed as described above.

Materials

The composition of the Krebs solution (pH 7.4) was (mM): NaCl 118.0, KCl 4.0, $MgSO_4$ 1.2, $CaCl_2$ 1.9, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.7. Additionally, propranolol (1 μM), a nonselective β-adrenoceptor antagonist, was present in all experiments and desmethylimipramine (100 nM) and corticosterone (40 μM), known to block neuronal and extraneuronal uptake of noradrenaline, were present in electrical stimulation experiments.

The following drugs were used: noradrenaline HCl, ouabain, veratridine, propranolol HCl, desmethylimipramine HCl, corticosterone and nifedipine (all from Sigma Chemical Co., St. Louis, MO, U.S.A.); ethylisopropylamiloride (Research Biochemical Inc. Natick, MA, U.S.A.); $^{22}Na^+$ and $^{45}Ca^{2+}$ (Amersham International, U.K.). All drugs, except corticosterone and nifedipine, were prepared as concentrated solutions in twice distilled water. Corticosterone and nifedipine were dissolved in 100% ethanol and dimethylsulphoxide, respectively, to obtain the desired concentrations. The final concentration of dimethylsulphoxide in the bathing solution did not exceed 0.1% and had no effect on the muscle contraction.

Data analysis

Data are expressed as means ± s.e.mean. Statistical significance was assessed by Student's t test and P values less than 0.05 were considered significant.

Results

Effect of ouabain on resting tone following repeated noradrenaline stimulation and electrical field stimulation

The exposure of human hyperplastic prostate to 1 μM ouabain for at least 100 min did not affect the resting tone (data not shown). In the absence of ouabain, tissues responded with reproducible contractions to repeated noradrenaline (10 μM) stimulation and full relaxation on washout (Figure 1a). However, when repeated noradrenaline stimulation was conducted in the presence of ouabain (1 μM), relaxation was progressively incomplete upon washout of the agonist and the resting tone increased, the magnitude rising with successive stimuli (Figure 1b and Table 1).

The nerve-mediated contractions were induced by electrical field stimulation and confirmed by their complete inhibition in the presence of tetrodotoxin (0.1 μM). As shown in Figure 1c, electrical field stimulation (80 V, 20 Hz, 0.2 ms) was conducted at 10 min intervals, reproducible peak contractions were observed and the resting tone was not affected in the absence of ouabain. However, in the presence of ouabain (1 μM), resting tone was progressively increased following electrical field stimulation; the tone was increased by 248 ± 19 mg after four episodes of stimulation in the presence of ouabain (Figure 1c).

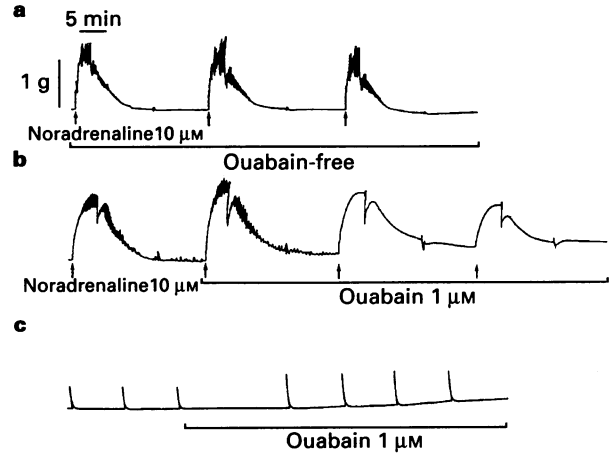

Figure 1 Traces of the resting tone and contractile responses of human hyperplastic prostate following repeated excitation. Tissues were repeatedly stimulated by noradrenaline (10 μM) in the absence (a) or presence (b) of ouabain (1 μM) or by electrical field stimulation (80 V, 20 Hz, 0.2 ms) in the absence or presence of ouabain (c). Artifacts in records were caused by solution changes.

Table 1 Resting tone between successive contractions induced by noradrenaline (10 μM) in the absence (control) or presence of ouabain (1 μM) in human hyperplastic prostate

	Resting tone (mg)		
Number of contractions	I	II	III
Control	0	0	0
Ouabain	184.0±19.1	267.3±18.5	378.3±26.8

Resting tone was measured after each noradrenaline addition. Noradrenaline-induced contractions were numbered for each noradrenaline addition. The resting tone before addition of the first noradrenaline was taken as zero. Values are means±s.e.mean of 6 determinations.

Additionally, the contractile responses to exogenously-applied noradrenaline and to electrical field stimulation were significantly augmented in the presence of ouabain as compared with its absence (Figure 1b, 1c) (1.42±0.09 g, $n=6$, $P<0.05$ and 0.68±0.07 g, $n=4$, $P<0.05$ as compared to control values of 1.10±0.07 g, $n=6$ and 0.48±0.04 g, $n=4$, respectively).

Effect of external calcium on elevation of the resting tone

The increased resting tone following repeated noradrenaline stimulation and electrical field stimulation was maintained well (for at least 30 min) in the presence of ouabain; this maintenance was not prevented by nifedipine (1 μM, data not shown) indicating that voltage-operated calcium channels were not involved; however, it was dependent on extracellular Ca^{2+}, as removal of Ca^{2+} from the bath induced a full relaxation of human prostate in both conditions, and subsequent restoration of calcium reintroduced an increase in resting tone (Figure 2).

Mechanism underlying the increased Na^+ influx

Figure 3 shows the effect of noradrenaline on the Na^+ uptake rate in human hyperplastic prostate. Noradrenaline significantly increased Na^+ uptake in a concentration-dependent manner and the submaximal concentration of noradrenaline (10 μM) was chosen to evaluate the effects of a number of agents on Na^+ uptake. Prazosin (0.1 μM) itself was ineffective in stimulating Na^+ uptake but it almost completely prevented noradrenaline from stimulating Na^+ accumulation in human hyperplastic prostate (Figure 4). Veratridine (50 μM), an alkaloid which activates voltage-dependent Na^+ channels in nerve and cardiac muscle (Catterall, 1980), induced a small but significant ($P<0.05$ as compared with control) increase of Na^+ uptake in human hyperplastic prostate. This effect was completely abolished by tetrodotoxin (1 μM). However, tetrodotoxin did not inhibit the Na^+ accumulation induced by noradrenaline (Table 2). In contrast, ethylisopropylamiloride, a specific inhibitor of Na^+/H^+ exchange, almost abolished the effect of noradrenaline on Na^+ transport (Table 2).

Effect of intracellular Na^+ loading on $^{45}Ca^{2+}$ uptake

To examine the effect of an increased $[Na^+]_i$ on $^{45}Ca^{2+}$ uptake, human hyperplastic prostates were stimulated with noradrenaline (10 μM) in the absence or presence of ouabain (1 μM). Figure 5 shows that tissues preincubated with ouabain demonstrated a significantly greater $^{45}Ca^{2+}$ uptake compared with control tissues ($P<0.05$). These results demonstrate that $^{45}Ca^{2+}$ uptake is enhanced by intracellular Na^+ loading. However, this effect on $^{45}Ca^{2+}$ uptake was not affected by nifedipine (1 μM) indicating that voltage-operated calcium channels were not involved.

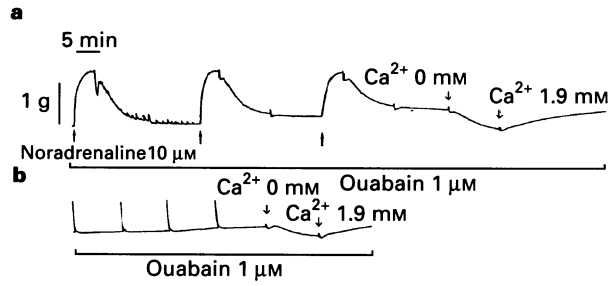

Figure 2 Effect of external Ca^{2+} on the resting tone following repeated excitation of human hyperplastic prostate. Tissues were treated with repeated noradrenaline (10 μM) stimulation (a) or electrical field stimulation (80 V, 20 Hz, 0.2 ms) (b) in the presence of ouabain (1 μM). After these treatments, external Ca^{2+} was removed from the medium and a full relaxation was observed and then 2 mM Ca^{2+} was reintroduced to the medium to induce an increase in resting tone. Artifacts in records were caused by solution changes.

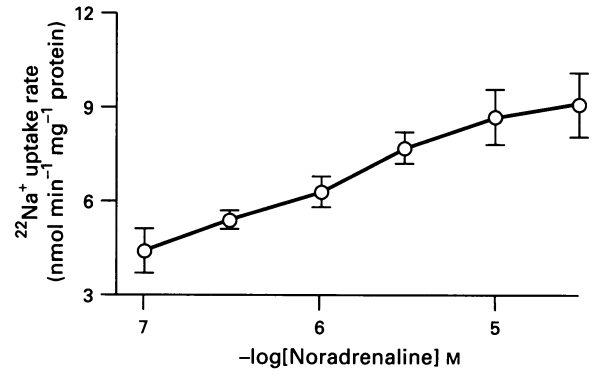

Figure 3 Effect of noradrenaline on $^{22}Na^+$ uptake rate in the presence of ouabain (1 μM) in human hyperplastic prostate. Values are means±s.e.mean of four determinations.

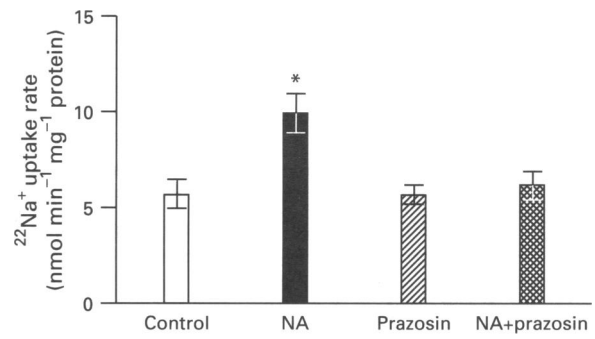

Figure 4 Effect of prazosin (0.1 μM) on noradrenaline (NA, 10 μM)-stimulated $^{22}Na^+$ uptake in the presence of ouabain (1 μM) in human hyperplastic prostate. Values are means±s.e.mean of four determinations. $^*P<0.01$ as compared with control.

Effect of intracellular Na^+ loading on $^{45}Ca^{2+}$ efflux

To examine the effect of intracellular Na^{2+} loading on $^{45}Ca^{2+}$ efflux, we studied noradrenaline-stimulated $^{45}Ca^{2+}$ efflux in the absence or presence of ouabain (1 μM). Figure 6 demonstrates that noradrenaline (10 μM)-stimulated Ca^{2+} efflux is significantly decreased by ouabain.

Table 2 The effect of tetrodotoxin (TTX) and ethylisopropylamiloride (EIPA) on the stimulation of Na^+ uptake by noradrenaline and veratridine in the presence of ouabain (1 μM)

Additions	$^{22}Na^+$ *uptake rate* (nmol min^{-1} mg^{-1} protein)
Control	5.68±0.72
Noradrenaline (10 μM)	9.83±1.01**
EIPA (100 μM)	4.33±0.66
Noradrenaline + EIPA	5.75±0.82
TTX (1 μM)	5.20±0.73
Noradrenaline + TTX	9.17±0.58**
Veratridine (50 μM)	7.42±0.30*
Veratridine + TTX	5.51±0.37

Values are expressed as means±s.e.mean of four determinations. *$P<0.05$ and **$P<0.01$ as compared with control.

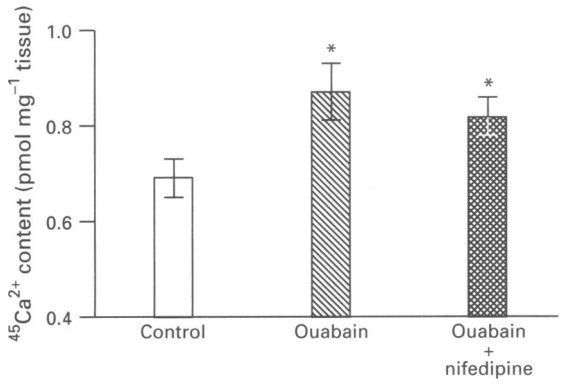

Figure 5 Effect of Na^+ loading on Ca^+ uptake. Human hyperplastic prostates were stimulated by noradrenaline (10 μM) in the absence (control, open column) or presence (hatched and cross-hatched columns) of ouabain (1 μM). Following the wash of agonist, tissues were incubated in the absence (open column, hatched column) or presence (cross-hatched column) of nifedipine (1 μM) for 15 min and 1 μCi ml^{-1} $^{45}Ca^{2+}$ was added as described in the Methods. Values are means±s.e.mean of seven determinations. *$P<0.05$ as compared with noradrenaline alone (control).

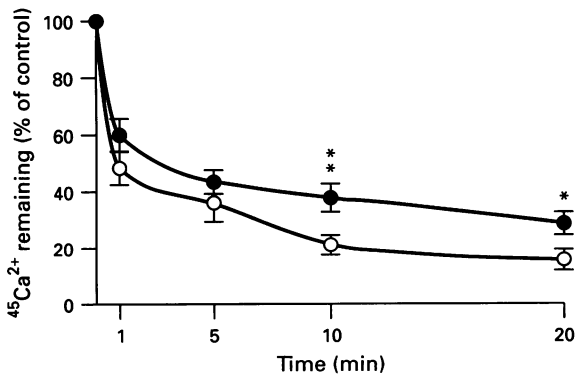

Figure 6 Effect of ouabain on noradrenaline-stimulated Ca^{2+} efflux. Human hyperplastic prostates were loaded with $^{45}Ca^{2+}$ for 3 h and washed. Tissues were exposed to noradrenaline (10 μM) in the first 5 min in the absence (○) or presence (●) of ouabain (1 μM) as described in the Methods. $^{45}Ca^{2+}$ content at control was 1.53±0.16 pmol mg^{-1} tissue ($n=9$). Results are expressed as percentage of Ca^{2+} remaining after the efflux period. Each point is the mean±s.e.mean of nine determinations. *$P<0.05$ and **$P<0.01$ as compared with the respective control.

Discussion

It has been suggested that nerve-mediated contractions in human prostate are controlled by the influence of the sympathetic nervous system acting via α_1-adrenoceptors (Caine, 1986; Guh *et al.*, 1995), and there is evidence to show that α_1-adrenoceptor stimulation is an important factor in the development of urinary obstruction in BPH (Yamada *et al.*, 1987; Kirby *et al.*, 1987; Jardin *et al.*, 1991). Both conditions of repeated noradrenaline stimulation and electrical field stimulation were carried out in this study. The contractile responses to exogenously-applied noradrenaline and to electrical field stimulation were significantly augmented in the presence of ouabain in human hyperplastic prostate. Similar effects were also observed in rat thoracic aorta and bovine tail artery (Ashida & Blaustein, 1987). It is likely that the results in the present study are due to the inhibition of the Na^+ pump, and then raising $[Ca^{2+}]_i$ and causing the sarcoplasmic reticulum to increase its store of Ca^{2+}. In addition, ouabain, at a concentration of 1 μM, exerted no effect *per se* on the resting tone of human hyperplastic prostate, but elicited an increase in testing tone under both conditions. It is known that noradrenaline, acting via the α_1-adrenoceptor, increases Na^+ influx into rabbit aorta (Aaronson & Jones, 1988) and canine arteries (Bukoski *et al.*, 1983). In an early study, Friedman & Allardyce (1962) showed that noradrenaline-induced tension development was parallel to uptake of Na^+ into canine femoral artery. In this study, we postulate that, in the presence of ouabain to inhibit Na^+-K^+ ATPase, the increased resting tone in human prostate following repeated noradrenaline stimulation and electrical field stimulation is a secondary response to increased Na^+ influx and/or decreased Na^+ efflux.

To investigate the mechanism of the increased tone, Ca^{2+} was removed from the medium after repeated noradrenaline stimulation and electrical field stimulation; the increased resting tone was fully relaxed in Ca^{2+}-free medium and recovered following subsequent restoration of calcium (Figure 2), revealing that the increased tone was dependent on extracellular Ca^{2+}. However, nifedipine was ineffective on this recovery of tension indicating that voltage-operated calcium channels were not involved. This study showed that Na^+ influx was a crucial factor for the increased tone following repeated excitation of human hyperplastic prostate in the presence of ouabain to block active Na^+ extrusion. It was of importance to investigate Na^+ influx after excitation of the tissues. In the present study, noradrenaline increased Na^+ influx in a concentration-dependent manner. Prazosin (0.1 μM) was virtually ineffective in stimulating Na^+ uptake but almost completely prevented noradrenaline from stimulating Na^+ accumulation. These results suggest that the increased Na^+ conductance by noradrenaline is a result of α_1-adrenoceptor activation.

There appear to be two distinct pathways for Na^+ uptake in human hyperplastic prostate. Veratridine caused a small but significant increase of Na^+ accumulation in the presence of ouabain (Table 2); tetrodotoxin completely blocked the effect by veratridine but had no effect on the action of noradrenaline (Table 2). Veratridine activates tetrodotoxin-sensitive Na^+ channels in nerves and in cardiac and skeletal muscle (Catterall, 1980) and also in human hyperplastic prostate in this study. Our finding suggests that noradrenaline activates a Na^+ uptake which is sensitive to ethylisopropylamiloride and these results indicate that Na^+/H^+ exchange is a major influx pathway of Na^+ in human hyperplastic prostate.

Extensive evidence indicates that various smooth muscles, including vascular and nonvascular smooth muscles, have a Na^+/Ca^{2+} exchange mechanism in their plasmalemma (Aickin *et al.*, 1984; Morel & Godfraind, 1984; Casteels *et al.*, 1985; Sheu & Blaustein, 1986) and experiments such as those of Aickin *et al.* (1984) on ureter provide convincing physiological evidence for Na^+/Ca^+ exchange in some smooth muscles. The Na^+/Ca^{2+} exchange system can move Ca^{2+} in either direction across the plasmalemma, depending upon the prevailing Na^+ electrochemical gradient. In addition, the contractile response

of smooth muscle to ouabain is ascribed to Ca^{2+} entry through the Na^+/Ca^{2+} exchange system as a consequence of Na^+ pump inhibition (Ozaki *et al.*, 1978; Woolfson *et al.*, 1990). Our results suggest that in the presence of ouabain to inhibit Na^+/K^+ ATPase, intracellular Na^+ loading by noradrenaline increases Ca^{2+} influx. It is unlikely that the ouabain-promoted Na^+-dependent Ca^{2+} influx was due to voltage-operated calcium entry since nifedipine was ineffective on this effect.

Ca^{2+} efflux in cardiac muscle has been suggested to be mediated predominantly by ATP-driven plasmalemmal pump (Ca^{2+}-ATPase) and Na^+/Ca^+ exchange (Scheid & Fay, 1984). At high $[Ca^{2+}]_i$ (e.g., following agonist stimulation), Na^+/Ca^{2+} exchange was activated and contributed to Ca^{2+} efflux. In this study, Ca^{2+} efflux was significantly elevated following noradenaline stimulation. However, in the presence of ouabain to promote elevation of $[Na^+]_i$, noradrenaline-stimulated Ca^{2+} efflux was diminished indicating that Na^+/Ca^{2+} exchange played a role in the regulation of $[Ca^{2+}]_i$. Additionally, Figure 5 and the accompanying results show that Ca^{2+} uptake was enhanced in the presence of ouabain; however, whilst noradrenaline-stimulated Ca^{2+} efflux is reduced by ouabain, it only just reaches significance after 10 min (see Figure 6) and the reduction is somewhat less pronounced than might be expected if Na^+/Ca^{2+} exchange was reversed. Presumably the reduced efflux alone could account for an increase in intracellular calcium without the need to invoke reversed Na^+/Ca^{2+} exchange.

In conclusion, ouabain, at a concentration insufficient for contraction, can elicit an increased tone in human hyperplastic prostate following repeated noradrenaline stimulation and electrical field stimulation; the increased tone is due to the increased Ca^{2+} entry and decreased Ca^{2+} extrusion through the Na^+/Ca^{2+} exchange system as a consequence of Na^+ pump inhibition.

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