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THE MECHANISM OF α-ADRENERGIC INHIBITION OF CATECHOLAMINE RELEASE

JACKIE COHEN, LEAH ECKSTEIN & YEHUDA GUTMAN

Department of Pharmacology, The Hebrew University, Hadassah School of Medicine, Jerusalem, Israel

1 The effect of α-adrenoceptor agonists on membrane adenosine triphosphatase (ATPase) activity was studied in membranes from the bovine adrenal medulla and the rat submaxillary gland.

2 α-Adrenoceptor agonists (10^{-7} to 10^{-5} M) enhanced significantly Na,K-ATPase activity but not Mg-ATPase activity in adrenal medulla. This effect was not observed in membranes from phaeochromocytoma. Phenylephrine (10^{-5} M), naphazoline (10^{-5} M) and clonidine (10^{-5} M) caused a significant increase of the activity of Na,K-ATPase (but not of Mg-ATPase) in the submaxillary gland. The enhancement became more prominent after ligature of the submaxillary duct but disappeared completely after superior cervical ganglionectomy. Thus, the effect of the α-adrenoceptor agonists was due to an action on adrenergic nerve terminals in the submaxillary gland.

3 Phenylephrine and naphazoline did not affect ^{45}Ca uptake but enhanced the rate of ^{45}Ca efflux from adrenal medullary slices *in vitro*.

4 Phenylephrine enhanced the rate of ^{45}Ca efflux from slices of submaxillary gland (with previous ligation of the duct); this was blocked by phentolamine and sympathetic denervation. Therefore phenylephrine was acting on the adrenergic nerve terminals.

5 It is suggested that the inhibition by α-adrenoceptor agonists of the exocytotic release of catecholamines from adrenergic nerve terminals and from chromaffin cells may be due to activation of the sodium pump, which results in enhancement of calcium efflux, causing a reduction of free intracellular Ca^{2+}.

Introduction

Catecholamine (CA) secretion from adrenergic nerve endings and from adrenal medulla can be inhibited by α-adrenoceptor agonists (Langer, 1974; Gutman & Bonnyaviroj, 1974; Boonyaviroj & Gutman, 1975). The inhibition of CA secretion affects both secretion of the amines and that of dopamine-β-hydroxylase in adrenergic nerve endings (Cubeddu & Weiner, 1975) and in the adrenal medulla (Boonyaviroj, Seiden & Gutman, 1977). Therefore, this is inhibition of an exocytotic process. However, the mechanism involved in this inhibitory action has not yet been elucidated. We have previously found that in the adrenal medulla *in vitro*, the inhibition of CA secretion by α-adrenoceptor agonists was abolished when the secretion of CA was stimulated either by ouabain (10^{-3} M) or by exposure to a potassium-free medium (Gutman & Boonyaviroj, 1977b). Under both these conditions the sodium pump was inhibited.

This finding indicated the possibility that the action of α-agonists involved activation of the sodium pump (Na,K-ATPase) (Gutman & Boonyaviroj, 1977a; Gutman, 1978). This possibility was corroborated by the finding that activation of the sodium pump by different means suppressed CA secretion from the adrenal medulla (Gutman & Boonyaviroj, 1977b).

Therefore, we have studied the effect of α-adrenoceptor stimulants on the membrane adenosine triphosphatase (ATPase) activity of the adrenal medulla. However, since the inhibition of CA release by α-agonists is observed in adrenergic nerve terminals, it seemed of importance to study whether the effect on Na,K-ATPase could also be demonstrated in nerve terminals. For this, the submaxillary gland of the rat was selected, since it has a dense adrenergic innervation, and, since by ligation of the duct, preferential degeneration of the glandular cells is obtained, while the nerve endings are spared. Furthermore, presynaptic α-adrenoceptor modulation of CA secretion has been demonstrated in the rat submaxillary gland by the use of phentolamine (Filinger, Langer, Perec & Stefano, 1978).

Finally, since the effect of the α-agonists is on an exocytotic process, i.e., a process involving Ca^{2+} (Douglas, 1968), the action on ^{45}Ca flux was also studied. It has been shown previously that the effec-

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tiveness of inhibition of CA release by α-agonists was affected by the concentration of calcium in the medium (Drew, 1978).

Methods

Animals

Male rats of the Hebrew University strain (200 to 250 g) were used. Immediately after dislocation of the neck, the submaxillary glands were removed through a midline cervical incision. The glands were placed on ice, dissected clean of connective tissue, cut into small pieces and homogenized (w/v = 1:10) in 0.32 M sucrose, containing 2 mM disodium edetate (EDTA), buffered to pH 7.4. Homogenization was carried out at 4°C, using a motor-driven Teflon plunger and a glass tube. The preparation procedure was described previously (Gutman, Hochman & Wald, 1973). After centrifugation at 7000 *g* for 10 min, the supernatant was centrifuged at 48,000 *g* for 30 min. The pellet was resuspended in 0.32 M sucrose with 0.67% desoxycholate (DOC) and incubated at room temperature for 30 min. After centrifugation at 5000 *g* for 10 min the supernatant was separated and centrifuged at 25,000 *g* for 30 min. The pellet was resuspended in 0.32 M sucrose, 1 ml per g of original tissue. This was immediately used as enzyme preparation or kept at −20°C for later studies. The activity of Na,K-ATPase under these conditions did not deteriorate for several months.

For preparation of enzyme from the adrenal medulla, bovine adrenals were obtained at the slaughterhouse, placed on ice and brought immediately to the laboratory. The adrenal medulla was dissected free of cortex and the same procedure as described above for preparation of Na,K-ATPase was followed.

Surgical procedures

Denervation of the submaxillary glands was performed unilaterally, under ether anaesthesia, by superior cervical ganglionectomy, under an illuminated magnifying lens. The submaxillary glands (on the denervated and on the control side) were removed 3 weeks after the surgical procedure.

Ligature of the duct of the submaxillary gland was performed under ether anaesthesia, through a midline cervical incision. The submaxillary glands were removed (on the ligated and control sides) 3 weeks after the surgical procedure. The combined procedure, ligature of the submaxillary duct and sympathetic denervation, was performed in one stage, under ether anaesthesia.

Assay of catecholamines

The submaxillary glands were homogenized in 0.4 M $HClO_4$ and left for 30 min. After centrifugation, the acid extract was passed sequentially through alumina and Biorex 70 columns, with appropriate pH adjustments, as described previously (Feuerstein, Boonyaviroj & Gutman, 1977). Catecholamine assay was carried out by the trihydroxyindole method, using an Aminco-Bowman Spectrofluorimeter, as described by Feuerstein *et al.* (1977).

Na,K-ATPase activity

The activity of Na,K-ATPase was assayed by measurement of the release of P_i from ATP during incubation at 37°C, in a medium composed of 100 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$ and 3 mM ATP, as previously described (Gutman & Beyth, 1969). To differentiate Na,K-ATPase activity from Mg-ATPase activity, incubation was carried out with or without addition of ouabain (10^{-3} M); alternatively, omission of K^+ from the incubation medium had the same effect as addition of ouabain. Protein was determined according to the method of Lowry, Rosebrough, Farr & Randall (1951). Inorganic phosphate was assayed by the method of Fiske & Subbarow (1925).

Calcium uptake and efflux from tissue slices in vitro

Slices of bovine adrenal medulla and of rat submaxillary gland (3 weeks after ligature of the duct) were placed in conical flasks (10 to 20 mg per slice, 3 to 6 slices in each 50 ml flask with 10 ml Locke solution). The slices were incubated for 20 min at 37°C in the presence of $^{45}CaCl_2$ (1 μCi per flask) with constant shaking. At the end of incubation, each slice was blotted on filter paper, rinsed in ice-cold medium and blotted again. The rinsing was carried out either in cold Locke solution or in cold Locke containing the drug to be used in the subsequent superfusion [phenylephrine (10^{-5} M) or phenylephrine and phentolamine (10^{-5} M)].

Each slice was then placed on filter paper at the bottom of a small funnel and was then superfused with the appropriate solution (Locke or Locke plus drug) at room temperature and at a constant rate of 0.2 ml/min (with a Harvard multichannel infusion pump). The outflow from the funnel was collected at intervals of 2 min for a total of 30 min. Each slice was then placed in a vial with 2 ml of double distilled water and extracted over-night in a shaking bath, at room temperature (17° to 20°C). More prolonged extraction yielded no additional efflux of $^{45}Ca^{2+}$ from the slice to the water.

The $^{45}Ca^{2+}$ content in the samples, collected during superfusion, and that extracted from the slice after

the end of the superfusion were determined (after addition of Instagel (Packard), 2.5 ml/0.5 ml sample) in a Packard Scintillation spectrometer. From the total amount of ^{45}Ca (efflux plus content of slice) the ^{45}Ca efflux was calculated for the duration of the superfusion. Results are expressed as the ^{45}Ca content of the slice at each time point as a percentage of the content at the beginning of superfusion (time 0).

Calcium uptake by slices of adrenal medulla was measured by incubation of slices (20 to 30 mg each) in Locke solution containing ^{45}Ca (1 μCi in 10 ml) for 20 min. For the effect of various drugs, the compounds were added to the incubation medium for the duration of the uptake study. At the end of incubation each slice was taken out, blotted, rinsed briefly in cold Locke solution, blotted again and then dissolved in 0.5 ml Soluene 350 (Packard) over-night in a shaking bath. After addition of 10 ml scintillation fluid, ^{45}Ca content was determined in a Packard spectrometer.

Phaeochromocytoma

Transplantable phaeochromocytoma was generously sent by Dr Shields Warren from New England Deaconess Hospital, Boston, Ma., U.S.A. as were the NEDH rats in which this tumour is transplanted.

Chemicals

Phenylephrine was purchased from Sigma, St Louis, Mo. U.S.A., naphazoline was a gift from Teva, Ltd., Jerusalem. Clonidine was kindly sent by Boehringer, Ingelheim. Phentolamine was a generous gift from CIBA, Basel. ATP was purchased from Merck, Darmstadt, Fed. Rep. Germany. $^{45}CaCl_2$ was obtained from New England Nuclear, Boston, Ma., U.S.A. (sp. act. 2.1 Ci/mmol).

Results

As can be seen in Figure 1, when plasma membrane ATPase was prepared from adrenal medulla, Mg-ATPase activity was not enhanced by noradrenaline, phenylephrine or naphazoline up to concentrations of 10^{-4} M. On the other hand, Na,K-ATPase was already significantly enhanced at concentrations of 10^{-6} M. The maximal effect was obtained at concentrations of 10^{-5} M to 2×10^{-5} M of the α-agonists and began to decline at 10^{-4} M. It should be noted that β-adrenoceptor agonists (isoprenaline and salbutamol both at 10^{-5} M) or β-adrenoceptor blockers (propranolol and practolol, both 10^{-6} M) had no significant effect on Na,K-ATPase activity. Antagonists at α-adrenoceptors (phentolamine 10^{-5} M, phenoxybenzamine, 10^{-5} M) blocked the effect of α-agonists and inhibited significantly ($P < 0.02$) the activation of

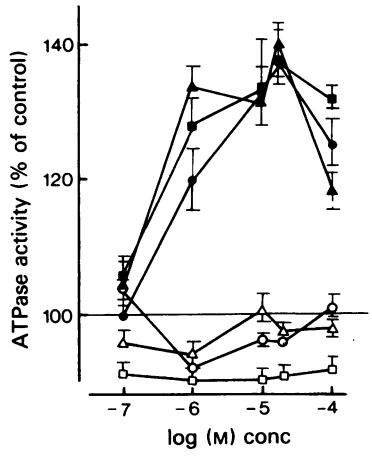

Figure 1 Effect of α-adrenoceptor agonists on Na, K-ATPase of adrenal medulla: (□ ■) noradrenaline; (○ ●) phenylephrine; (△ ▲) naphazoline. Open symbols: Mg-ATPase activity; filled symbols: Na,K-ATPase activity. Each point is the mean of 10 experiments; vertical lines show s.e. mean. The activity of control adrenal medula was: Na,K-ATPase = 39.7 ± 3.4 μmol Pi/mg protein and Mg-ATPase = 54.3 ± 1.7 μmol Pi/mg protein, both per hour (n = 10).

Na,K-ATPase of adrenal medulla (not shown in figure). In the presence of 10^{-5} M phentolamine, the dose-response curve for naphazoline was shifted to the right by a dose-ratio of 15.

Ouabain at concentrations lower than 10^{-9} M caused significant enhancement of Na,K-ATPase activity of the adrenal medulla (Figure 2). When the release of CA was studied, 10^{-10} M ouabain decreased the secretion rate from 0.48 ± 0.02 μg/10 min to 0.33 ± 0.01 μg/10 min while at 10^{-3} M ouabain, CA secretion was increased from 0.48 ± 0.02 to 0.74 ± 0.02 μg/10 min.

Effect of α-adrenoceptor agonists on Na,K-ATPase of submaxillary gland

Figure 3 shows that phenylephrine (10^{-5} M) caused a significant enhancement of Na,K-ATPase, prepared from the submaxillary gland but had no effect on Mg-ATPase activity (left part of Figure 3). Following denervation of the submaxillary gland, the effect of phenylephrine on Na,K-ATPase activity was abolished (Figure 3). This suggested that the enhancement of Na,K-ATPase was due to an effect on adrenergic nerve terminals. Table 1 shows that noradrenaline content of the submaxillary gland was substantially lowered by denervation. However, since nerve terminals constitute only a very small fraction of the

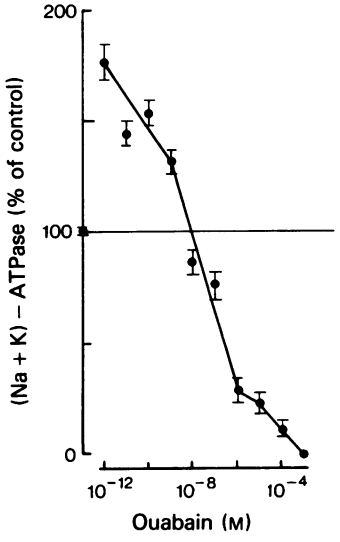

Figure 2 Biphasic effect of ouabain on Na,K-ATPase activity of adrenal medulla. Each point is the mean of 10 experiments; vertical lines show s.e. mean.

tissue, the activity of Na,K-ATPase was not significantly changed after denervation by superior cervical ganglionectomy (Table 1).

Following ligation of the submaxillary duct there was considerable degeneration of the glandular cells, as reflected in a substantial reduction of Na,K-ATPase (Table 1). However, adrenergic nerve terminals were not affected: noradrenaline content of the submaxillary gland was unchanged (Table 1). Na,K-ATPase prepared from the ligated gland was enhanced more dramatically by phenylephrine (10^{-5} M) than the enzyme from control glands (Figure 3).

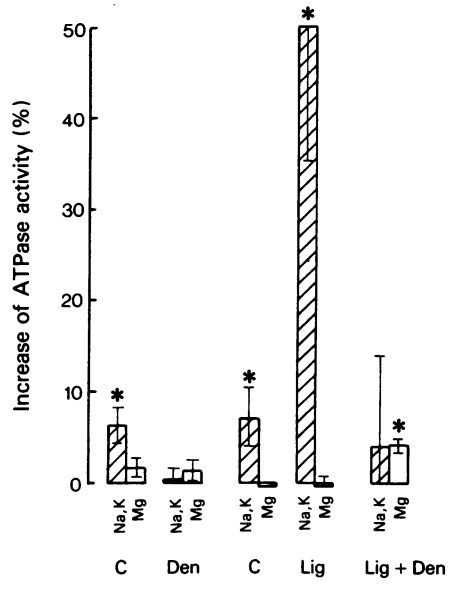

Figure 3 Effect of phenylephrine on Na,K-ATPase activity in the submaxillary gland. Na,K = Effect of 10^{-5} M phenylephrine on Na,K-ATPase activity. Mg = Effect of 10^{-5} M phenylephrine on Mg-ATPase activity. From left to right: C = control submaxillary glands (n = 28); Den = submaxillary glands taken from same rats as C, denervated by superior cervical ganglionectomy (n = 28). C = control submaxillary glands (n = 32); Lig = submaxillary glands from same rats as C following ligature of the major salivary duct (n = 32). Lig + Den = submaxillary glands following superior cervical ganglionectomy and ligation of salivary duct (n = 38). Vertical bars = s.e. mean.

* $P < 0.01$ compared to ATPase activity in the absence of phenylephrine.

Table 1 Effect of various surgical procedures on noradrenaline content and Na,K-ATPase activity of submaxillary gland

	Control	*Denervated*	*Ligature*	*Ligature plus denervation*
Noradrenaline (μg/gland)	0.19 ± 0.01 (n = 11)	0.04 ± 0.006 (n = 6)	0.18 ± 0.01 (n = 8)	0.07 ± 0.006 (n = 3)
Na,K-ATPase (μmol Pi/mg prot × h)	28.6 ± 1.4 (n = 28)	34.6 ± 2.6 (n = 28)	10.3 ± 0.8 (n = 34)	6.1 ± 0.4 (n = 38)

n = number of experiments. Results expressed as mean ± s.e. mean.
Ligature = submaxillary glands taken three weeks after ligation of the major salivary duct; Denervated = sympathetically denervated glands by superior cervical ganglionectomy.
For noradrenaline content, 'denervated' compared to 'control' and 'denervation + ligature' compared to 'ligature', $P < 0.01$.
For Na,K-ATPase activity: ligature vs. control; ligature + denervation vs. ligature, $P < 0.01$.

Superior cervical ganglionectomy in the ligated submaxillary gland caused reduction of noradrenaline content (Table 1), and a significant reduction of Na,K-ATPase activity (Table 1), suggesting that this decrease was due to degeneration of the adrenergic nerve terminals. Na,K-ATPase prepared from the denervated-ligated submaxillary gland was not significantly enhanced by phenylephrine (Figure 3).

Activation of Na,K-ATPase from submaxillary gland was also observed with two other α-adrenoceptor agonists, naphazoline and clonidine, with peak effect at 10^{-5} M (not shown in figure); the ED_{50} for naphazoline 10^{-7} M. Furthermore, the enhancement caused by phenylephrine (10^{-5} M) was inhibited by the α-adrenoceptor antagonists, phentolamine (10^{-5} M, $P < 0.01$) and phenoxybenzamine 10^{-5} M ($P < 0.05$).

Effect of phenylephrine on Na,K-ATPase after in vivo *exposure to noradrenaline*

In rats bearing a transplantable phaeochromocytoma, which secretes predominantly noradrenaline (Chalfie & Perlman, 1976), Na,K-ATPase activity was assayed in the tumour itself and in the submaxillary gland of the tumour-bearing rats. Chronic exposure to noradrenaline, secreted by the tumour *in vivo*, caused complete elimination of the effect of phenylephrine (10^{-5} M) on the activity of Na,K-ATPase in the submaxillary gland. Also in the membrane preparation of the phaeochromocytoma, no activation of Na,K-ATPase could be observed in the presence of phenylephrine. This was in contrast to the enhancement observed in the adrenal medullary chromaffin cells of Na,K-ATPase in the presence of phenylephrine (Figure 1). It is noteworthy that phenylephrine also had no inhibitory effect on catecholamine release from phaeochromocytoma (unpublished observation in our laboratory), in contrast to the significant inhibition of CA secretion from the adrenal medulla: from 1.33 ± 0.04 μg/gland released by 10^{-4} M acetylcholine to 0.75 ± 0.02 μg/gland when 10^{-5} M phenylephrine was present.

Effect of various agents on ^{45}Ca uptake by slices of adrenal medulla

When the uptake of ^{45}Ca by bovine adrenal medulla slices *in vitro* was studied, a significant increase in uptake was observed in the presence of acetylcholine (10^{-4} M) or in a high potassium medium (56 mM), ($P < 0.01$). Various agents which inhibit CA secretion (phenylephrine 10^{-5} M, naphazoline 10^{-5} M, diphenylhydantoin 10^{-4} M and low concentration of ouabain, 10^{-10} M) had no significant effect on ^{45}Ca uptake ($P < 0.05$).

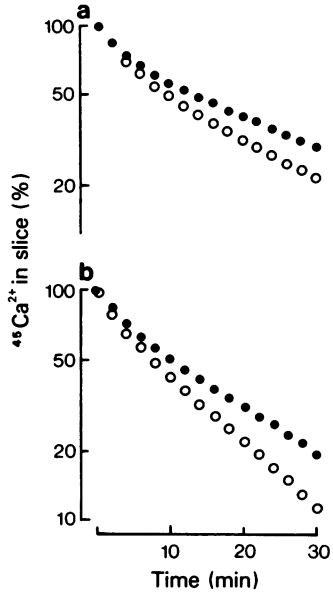

Figure 4 Effect of naphazoline (a) and phenylephrine (b) on ^{45}Ca efflux from slices of adrenal medulla *in vitro*. In (a) and (b), (●) = efflux from control slices (superfused with Locke solution). In (a), (○) = efflux during superfusion with Locke solution containing 10^{-5} M naphazoline. In (b), (○) = efflux during superfusion with Locke solution containing 10^{-5} M phenylphrine. Each point is the mean of 10 experiments. In (a), each point of naphazoline line from 20 to 30 min significantly different ($P < 0.05$) from corresponding point of control. In (b) each point of phenylephrine line, from 18 min to 30 min significantly different ($P < 0.05$) from corresponding point of control.

Effect of phenylephrine on ^{45}Ca efflux

When the rate of $^{45}Ca^{2+}$ efflux from slices of adrenal medulla, preloaded with $^{45}Ca^{2+}$, was followed during superfusion with Locke solution, addition of phenylephrine (10^{-5} M) to the superfusion fluid caused enhancement of the $^{45}Ca^{2+}$ efflux rate (Figure 4). A similar effect was obtained with naphazoline (10^{-5} M) (Figure 4). α-Adrenoceptor blockers (phentolamine 10^{-6} M and phenoxybenzamine 10^{-6} M) caused the opposite effect, i.e. reduced the rate of ^{45}Ca efflux.

A similar experiment was carried out with slices of rat submaxillary gland (3 weeks after ligation of the duct of the gland). As can be seen in Figure 5, the rate of efflux of $^{45}Ca^{2+}$ in superfused slices, preloaded with $^{45}Ca^{2+}$, was accelerated when phenylephrine (10^{-5} M) was added to the superfusion medium. Furthermore, when phentolamine (10^{-5} M) and phenylephrine (10^{-5} M) were present simultaneously in the superfusion fluid, the acceleration of $^{45}Ca^{2+}$

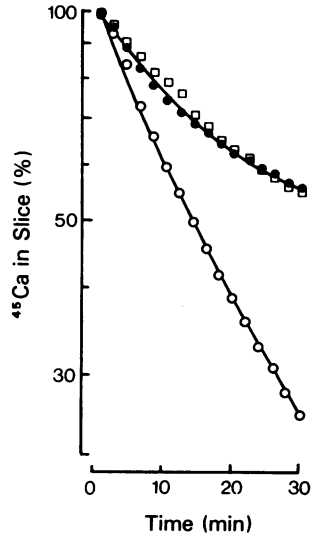

Figure 5 Effect of phenylephrine on ^{45}Ca efflux from slices of submaxillary gland *in vitro*. The salivary glands were ligated (duct) three weeks before the experiment. (●) = Efflux of ^{45}Ca from control slices (superfused with Locke solution); (○) = efflux during superfusion with Locke solution containing 10^{-5} M phenylephrine (□) = efflux during superfusion with Locke solution containing 10^{-5} M phenylephrine and 10^{-5} M phentolamine. Each point is the mean of 6 experiments. Each point of phenylephrine line (○), from 8 min of the superfusion, is significantly different ($P < 0.01$) from the corresponding time point of control and of phenylephrine plus phentolamine lines.

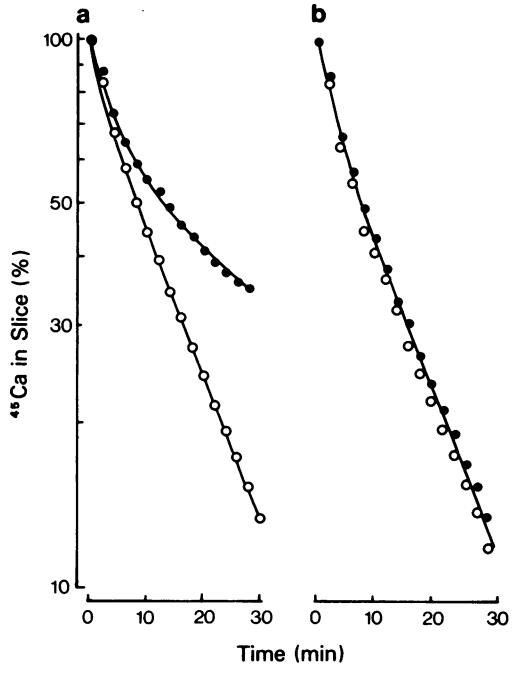

Figure 6 Effect of phenylephrine on ^{45}Ca efflux from slices of (a) innervated and (b) denervated submaxillary glands *in vitro*. All salivary glands were duct-ligated three weeks before the experiments. The denervated glands were also sympathectomized by superior cervical ganglionectomy simultaneously with the duct ligation. In (a), (●) = efflux of ^{45}Ca from control slices (superfused with Locke solution); (○) = efflux of ^{45}Ca from slices superfused with Locke solution containing 10^{-5} M phenylephrine. Each point is the mean of 6 experiments. Each point of phenylephrine lines, from 12 min onwards, is significantly different ($P < 0.05 - P < 0.01$) from the corresponding time point of control line. In the denervated glands (b), there was no significant difference between control (●) and phenylephrine-treated (○) slices at any point.

efflux by phenylephrine was completely abolished (Figure 5).

When the duct-ligated gland was also denervated (sympathetic denervation by superior cervical ganglionectomy), the effect of phenylephrine (10^{-5} M) on ^{45}Ca efflux was completely abolished (Figure 6, compare (b) and (a)).

Discussion

The present paper confirms and extends our previous finding on activation of adrenal medullary Na,K-ATPase by α-adrenoceptor agonists (Gutman & Boonyaviroj, 1977a). Enhancement of brain Na,K-ATPase activity by adrenoceptor agonists has been described by several authors (Yoshimura, 1973; Godfraind, Koch & Verbeke, 1974; Lee & Phillis, 1977). The characteristics of adrenal medullary chromaffin cells resemble those of adrenergic nerve endings in several respects: exocytotic release takes place from these cells, the release of CA is modulated by the same agents, as in adrenergic nerve endings (α-adrenoceptor-agonists, β-adrenoceptor-agonists, prostaglandin E: Gutman, 1978). Therefore, it seemed possible that the effect on Na,K-ATPase would also be found in adrenergic nerve endings.

The submaxillary gland is composed mainly of glandular cells and is innervated by adrenergic nerve terminals (as well as cholinergic innervation). α-Adrenoceptor agonists affect salivary secretion directly; therefore, the glandular cells may be assumed to have α-receptors. The adrenergic nerve endings are also assumed to carry presynaptic α-receptors (Filinger *et al.*, 1978). When the presynaptic α-receptors are activated, release of noradrenaline is inhibited (Langer, 1974). Secretion of saliva by activation of α-adrenoceptors, on the glandular cells depends on increase of

cellular Ca^{2+} (Putney, 1976). An increase of Ca_i^{2+} would result from inhibition of Na,K-ATPase in gland cells rather than from activation of the sodium pump. On the other hand, in adrenergic nerve endings, activation of α-receptors, causes inhibition of exocytotic secretion of CA (Cubeddu & Weiner, 1975; Boonyaviroj *et al.*, 1977). It would be reasonable to assume that decreased cellular Ca^{2+} could cause such an effect, i.e. inhibition of exocytosis. Therefore, activation of α-adrenoceptors would result in opposite effects in adrenergic nerve terminals and in glandular (post-synaptic) α-adrenoceptors, i.e. decreased and increased cellular Ca^{2+}, respectively.

The experiment illustrated in Figure 3 shows that the enhancement of Na,K-ATPase in the submaxillary gland by α-agonists was observed only under conditions where adrenergic terminals were present: the effect was abolished after superior cervical ganglionectomy. Furthermore, the effect was accentuated when glandular cells had degenerated (ligation of duct), suggesting that the enhancement of Na,K-ATPase activity by α-adrenoceptor agonists was not due to an effect on membranes originating from salivary gland cells but rather to an effect on membranes from nerve endings. A recent report on Na,K-ATPase in subcellular fractions of brain also points to activation by α-receptors (Lee & Phillis, 1977).

Experimental evidence suggests, therefore, that activation of presynaptic and postsynaptic α-receptors initiate different mechanisms: while activation of Na,K-ATPase is specific for pre and not for postsynaptic effects (Figure 3), postsynaptic action of α-receptors causes changes in ionic permeability, particularly to potassium and calcium (Jenkinson, Haylett & Koller, 1978). This difference may lead to possible specific interference (by drugs) with post and presynaptic α-adrenoceptor action, respectively.

Corroborating this line of reasoning, agents unrelated structurally to CA but having the same action on Na,K-ATPase also mimicked the effect of α-adrenoceptor stimulants on CA release, i.e. caused inhibition of release. Thus, we have previously shown that diphenylhydantoin inhibited CA secretion from the adrenal medulla and concomitantly caused enhancement of Na,K-ATPase activity (Gutman & Boonyaviroj, 1977b). Similarly, a low concentration of ouabain (10^{-10} M) inhibited CA release from adrenal medulla and increased Na,K-ATPase activity. As seen in Figure 2, a dose-response curve for ouabain in the adrenal medulla confirmed the enhancement of Na,K-ATPase activity at concentrations below 10^{-9} M. Activation of Na,K-ATPase or of the sodium pump by low concentrations of cardiac glycosides ($<10^{-8}$ to 10^{-9} M) has been shown in a variety of tissues: rabbit gall bladder (Van Os & Slegers, 1976), toad bladder (McClane, 1965) chicken and dog kidney (Palmer & Nechay, 1964; Nachmod & Walser, 1966), cardiac muscle (LaBella, Bihler & Kim, 1979).

On the other hand, it is well known that inhibition of Na,K-ATPase, by omission of potassium from the medium or by high concentrations of cardiac glycosides, increase CA secretion (Banks, 1967; Gutman & Boonyaviroj 1977a,b). Since inhibition of the sodium pump enhances CA release probably through increased intracellular Ca^{2+} (Ca_i), it would seem plausible that activation of the sodium pump should inhibit CA release through decreased Ca_i (Gutman, 1978). Reduced Ca_i should inhibit exocytosis, as was indeed the effect of α-adrenoceptor agonists: inhibition of release of dopamine-β-hydroxylase (Cubbedu & Weiner, 1975; Boonyaviroj *et al.*, 1977).

Activation of the sodium pump should lead to lowering of intracellular Na (Na_i). The reduced Na_i could affect Ca_i either through a common membrane carrier, as suggested by Baker (1978) or through decreased Ca^{2+} release from intracellular storage sites. Recently it has been shown that the intracellular sodium concentration can regulate release of Ca from mitochondria (Carafoli & Crompton, 1978).

The experiments on the effect of α-adrenoceptor agonists on $^{45}Ca^{2+}$ efflux (Figures 4, 5 and 6) support the hypothesis of increased outward calcium movement, suggested by Baker (1978). Thus, activation of the sodium pump reduces the intracellular sodium concentration. The lowered Na_i could reduce exchange of extracellular calcium (Ca_0) for Na_i and may, therefore, provide a pathway for net reduction of Ca_i due to calcium efflux.

The relationship between activation of Na,K-ATPase and increased $^{45}Ca^{2+}$ efflux has more than one explanation and awaits further experimentation.

The experiments described here were carried out in order to identify the molecular events following interaction of the α-adrenoceptor agonists and antagonists with their receptors; therefore, no specific characterization of the subtype of α-receptors (α_1 or α_2) was performed. Differentiation of pre from postsynaptic effects was achieved mainly by use of the surgical approach, i.e. sympathetic denervation and duct-ligation of the submaxillary gland.

This paper is part of an M.Sc. thesis of L.E. and part of a Ph.D. thesis of J.C.

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