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The action of excess potassium and calcium on ouabain-evoked [^{3}H]-noradrenaline release from the rabbit pulmonary artery

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1 [^{3}H]-noradrenaline ([^{3}H]-NA) release from the main pulmonary artery of the rabbit has been measured in the presence of neuronal (cocaine, 3×10^{-5} M) and extraneuronal (corticosterone, 5×10^{-5} M) uptake blockers.

2 Removal of K from the external medium increased the [^{3}H]-NA release. In the absence of external K, ouabain (10^{-4} M) further enhanced the neurotransmitter release. The 'K-free' stimulated [^{3}H]-NA release was inhibited by an increase of external Ca (7.5 mM), an action antagonized by ouabain.

3 After preperfusion of the preparations for 30 min with either excess K (23.6 mM) or excess Ca (7.5 mM), the ouabain-stimulated [^{3}H]-NA release was inhibited by about 50%; the rates of inhibition did not differ significantly from each other. However, the characteristic initial delay before ouabain-evoked neurotransmitter release was shortened in excess K, and prolonged in excess Ca-containing solution.

4 When both excess K and Ca were applied together 30 min before ouabain perfusion, the action of ouabain in releasing neurotransmitter was also inhibited but the rate of inhibition did not differ significantly from that seen when K or Ca were applied separately. The action of K in shortening the initial delay was partly antagonized by Ca.

5 Excess Ca antagonized the inhibition of ouabain-stimulated [^{3}H]-NA release caused by excess K when Ca and ouabain were applied together after 30 min preperfusion with excess K-containing solution. Again excess Ca failed to inhibit the ouabain-evoked neurotransmitter release if ouabain and excess K were applied together after excess Ca preperfusion (30 min). In both cases the initial delay of ouabain action was greatly shortened.

6 The results suggest a Na-Ca competition at the external activation site of the nerve terminal sodium-pump similar to that of Na-K competition. Furthermore it seems that there is a sort of K-Ca competition as well, suggested by the finding that excess Ca prevented the inhibition caused by excess K of ouabain-evoked noradrenaline release and *vice versa*.

Introduction

It has been shown that inhibition of the Na-pump either by K-removal from the external medium or ouabain application increases both the acetylcholine and noradrenaline release from presynaptic nerve terminals (Birks & Cohen, 1968; Paton *et al.*, 1971; Vizi, 1972; 1977; Baker & Crawford, 1975; Bonaccorsi *et al.*, 1977; Nakazato *et al.*, 1978; Vizi *et al.*, 1982; 1984; Powis, 1983; Török *et al.*, 1984). In the noradrenergic nerves of the main pulmonary artery of the rabbit the action of ouabain is Na-dependent (Török *et al.*, 1984) as in other cell membranes (Baker, 1968; Baker & Manil, 1968; Baker & Willis, 1970; 1972; Baker & Crawford, 1975; Akera, 1977), and can be antagonized by external K (Török *et al.*, 1984). The inhibitory action of external K is the result of the well known Na-K competition at the external activation site of the Na-pump (Glynn, 1964; Matsui & Schwartz, 1968; Baker & Willis, 1970; 1972; Lindenmayer & Schwartz, 1973; Schwartz *et al.*, 1975; Schwartz, 1976). On the other hand Repke (1963) and Schön *et al.* (1972) have suggested an antagonism between Na and Ca, which possibly regulates the activity of Na,K-ATPase. Baker & Crawford (1975) have shown that external Ca removal shortens the initial delay of

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ouabain-evoked acetylcholine release from the motor nerve terminal. In Na-pump inhibited Purkinje fibres, excess Ca decreased the internal Na activity (Deitmer & Ellis, 1978) and in myocardial cells excess Ca inhibited both the binding and uptake of ouabain (Meldgaard *et al.*, 1981). Furthermore Powis *et al.* (1983) have shown that Ca stimulates the neuronal Na,K-ATPase. In addition Schwartz (1976) has suggested that Ca and K may have some relationship to the K-activation site of the Na-pump, and that Ca can reduce the amount of K required to activate the Na-pump.

In a preceding paper it has been found that excess Ca inhibits the 'K-free' stimulated NA release from pulmonary arteries and further delays the neurotransmitter releasing action of ouabain (Török *et al.*, 1984).

Here we have investigated the inhibition by excess K and Ca of ouabain-evoked [^{3}H]-NA release from the arteries. It was found that excess Ca, like excess K, inhibits the transmitter releasing action of ouabain and causes a prolongation of the initial delay which precedes the NA releasing action of the cardiac glycoside. Excess Ca also inhibited the 'K-free' stimulated NA release, an action antagonized by ouabain. Since the action of ouabain is Na-dependent, the results suggest a Na-Ca competition at the external activation site of the Na-pump similar to the Na-K competition. Furthermore, an unexpected observation suggests a form of K-Ca competition as well.

Methods

The experiments were carried out in the isolated main pulmonary artery of the rabbit (Starke *et al.*, 1974). Rabbits of either sex (2–3 kg) were stunned by a blow on the head. The main pulmonary artery was dissected and placed in normal Krebs solution which contained the monoamine oxidase inhibitor pargyline (1.2×10^{-4} M) and which was fully equilibrated with 5% CO_2, 95% O_2. Table 1 shows the composition of normal and modified Krebs solutions used throughout.

Measurement of [^{3}H]-noradrenaline release

The method has been described previously (Borowski *et al.*, 1977; Endo *et al.*, 1977; Török *et al.*, 1984). Briefly, after the preparation had been placed in Krebs solution, 25 µl [^{3}H]-NA was added to the incubation solution (final concentration of [^{3}H]-NA: $4.2–4.6 \times 10^{-7}$ M) for 45 min (pargyline and the NA stabilizing agents ascorbic acid, 3×10^{-4} M and disodium edetate (Na_2EDTA), 3×10^{-5} M were present). The temperature of the Krebs solution was 37°C. Subsequently the preparation was suspended in an organ bath (capacity: 2 ml) and superfused at a rate of 8 ml min^{-1} with 800 ml of medium containing the neuronal uptake blocker cocaine (3×10^{-5} M) instead of pargyline. At the end of the washing period the flow rate was changed to 4 ml min^{-1} and the extraneuronal uptake blocker corticosterone (5×10^{-5} M) was also added to the Krebs solution. Endo *et al.* (1977) have found that in the presence of uptake blockers, 86% of

Table 1 Compositions of normal and modified Krebs solutions used in experiments (mmol litre^{-1})

		Solutions				
Ions	Normal (1)	(2)	(3)	(4)	(5)	(6)
Na^+	137.4	138.6	138.6	119.7	137.4	119.7
K^+	5.9	—	—	23.6	5.9	23.6
Ca^{2+}	2.5	2.5	7.5	2.5	7.5	7.5
Mg^{2+}	1.2	1.2	1.2	1.2	1.2	1.2
Cl^-	122.1	117.4	127.4	122.1	132.1	132.1
HCO_3^-	25.0	25.0	25.0	25.0	25.0	25.0
$H_2PO_4^-$	1.2	1.2	1.2	1.2	1.2	1.2
SO_4^{2-}	1.2	1.2	1.2	1.2	1.2	1.2
Glucose	11.5	11.5	11.5	11.5	11.5	11.5

All of the solutions contained ascorbic acid (3×10^{-4} M), Na_2EDTA (3×10^{-5} M), cocaine (3×10^{-5} M) and corticosterone (5×10^{-5} M). The modified Krebs solutions are as follows: (2), 'K-free'; (3), 'K-free' and 3 × Ca; (4), 4 × K; (5), 3 × Ca; (6), 4 × K and 3 × Ca. When K was removed, twice distilled water was used for preparing Krebs solution. In 'K-free' solution KCl (4.7 mM) was simply omitted without ionic compensation, however, KH_2PO_4 (1.2 mM) was substituted by an equimolar concentration of NaH_2PO_4. In excess K (23.6 mM)-containing solutions, the NaCl was reduced. When excess Ca (7.5 mM) was used, NaCl was not reduced. At the beginning of the experiments for dissection and loading the pulmonary arteries in radiolabelled noradrenaline, pargyline (1.2×10^{-4} M) was present in normal solution instead of cocaine and corticosterone.

liberated NA is unmetabolised. On the basis of this assumption, and knowing the specific activity of [^{3}H]-NA, we calculated the outflow of labelled neurotransmitter in pmol 6 min^{-1} (Endo *et al.*, 1977). Tetrodotoxin (10^{-7} M) was effective in abolishing the tritium release evoked by field stimulation (2 Hz, 360 pulses) indicating the nervous origin of liberated NA. The superfusate was fractionated and the radioactivity was determined in a Beckman (LS-9000) liquid scintillation spectrometer.

Drugs, statistics

The following drugs were used: (−)-[7-^{3}H]-noradrenaline, specific activity: 35.9–40.0 Ci mmol^{-1} (Radiochemical Centre, Amersham); pargyline hydrochloride (Serva); cocaine hydrochloride (Merck); corticosterone (Fluka); ouabain (Calbiochem); tetrodotoxin (Calbiochem); ascorbic acid (EGA); disodium edetate (Na_2EDTA, Aldrich-Europe). The drugs were dissolved in Krebs solution. Corticosterone was dissolved in propylene glycol (final concentration 0.05%). All of the chemicals used to prepare Krebs solution were of analytical grade. Mean values ± s.e.mean are given. Significances of difference were calculated by Student's *t* test; *n* is the number of experiments.

Results

Inhibition of 'K-free' stimulated [^{3}H]-noradrenaline release by excess Ca and its reversal by ouabain

Removal of K from the Krebs solution significantly increased the [^{3}H]-NA release from pulmonary arteries (Figure 1a) as has been shown previously (Török *et al.*, 1984). After 180 min Na-loading, ouabain (10^{-4} M)

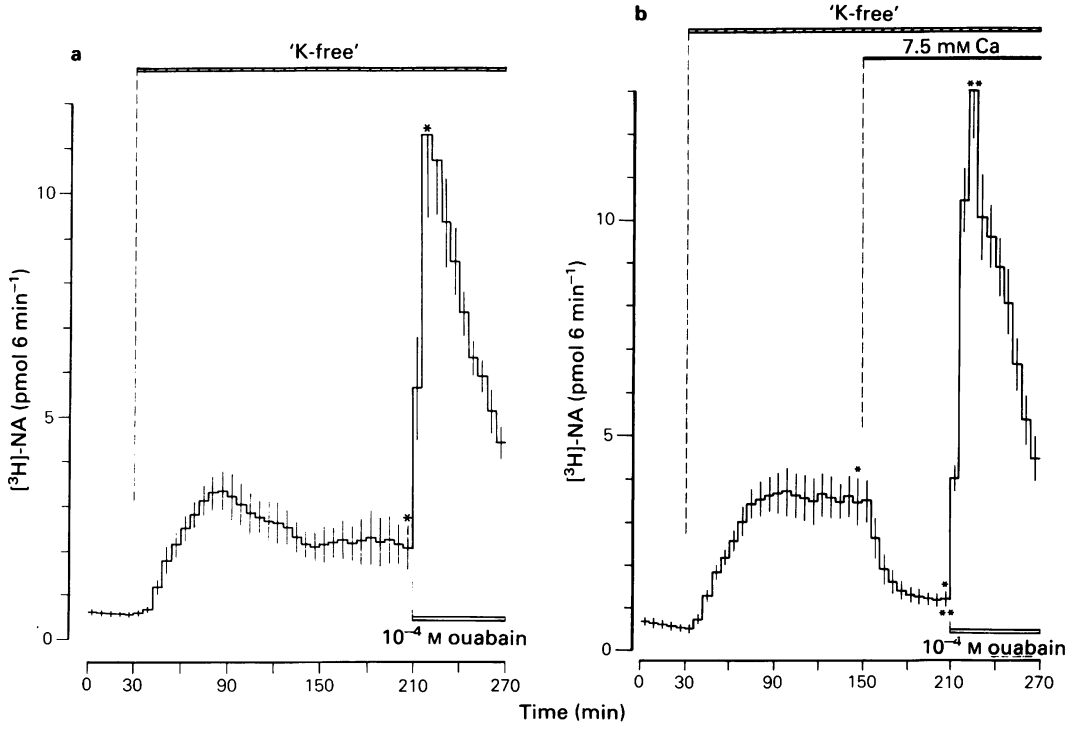

Figure 1 [^{3}H]-noradrenaline release evoked by Na-pump inhibition and the effect of excess Ca (7.5 mM) in the isolated main pulmonary artery of the rabbit in the presence of cocaine (3×10^{-5} M) and corticosterone (5×10^{-5} M). Ordinate scale: [^{3}H]-NA outflow, expressed in pmol 6 min^{-1}; abscissa scale: time (min). (a) In 'K-free' Krebs solution the [^{3}H]-NA release increased. After 180 min, ouabain (10^{-4} M) further enhanced neurotransmitter release; s.e.means of five identical experiments are shown by vertical lines. Significant differences as indicated (*$P < 0.01$). (b) After 120 min 'K-free' treatment, 7.5 mM Ca was applied for 60 min which inhibited the release of [^{3}H]-NA. In the absence of external K and in the presence of excess Ca, 10^{-4} M ouabain immediately enhanced the [^{3}H]-NA release. S.e.means of six identical experiments shown by vertical lines. Significant differences between values: *$P < 0.02$; **$P < 0.001$.

significantly further enhanced the neurotransmitter release (from 2.04 ± 0.49 to 11.29 ± 1.87 pmol $6\,min^{-1}$; $n = 5$; $P < 0.01$) without the initial long delay (compare with Figure 2). However, the amount of released NA in response to ouabain in 'K-free' solution was decreased. The rapid onset of the neurotransmitter releasing action of ouabain in 'K-free' solution confirms the well-known Na-K competition at the external activation site of the Na-pump (cf. Baker & Willis, 1970; 1972) and the Na-dependent binding of ouabain (Baker & Connelly, 1966; Baker, 1968; Baker & Manil, 1968; Baker & Crawford, 1975; Schwartz *et al.*, 1975). Excess Ca (7.5 mM) inhibited the 'K-free' stimulated NA release from pulmonary arteries (Figure 1b), i.e. after 120 min of K-removal, 7.5 mM Ca (3 times normal) was introduced into the perfusion solution for 60 min and this decreased the [^{3}H]-NA release from 3.44 ± 0.50 to 1.20 ± 0.20 pmol $6\,min^{-1}$ ($n = 6$). The difference was significant at a level of 0.02. In the absence of external K and in the presence of excess Ca, ouabain (10^{-4} M) significantly enhanced the labelled neurotransmitter release to 12.91 ± 1.20 pmol $6\,min^{-1}$ ($P < 0.001$; Figure 1b).

[^{3}H]-noradrenaline releasing action of ouabain in excess potassium- and/or excess calcium-containing solutions

In normal external ionic environments 10^{-4} M ouabain transiently increased the neurotransmitter release after an initial delay (Figure 2a). A similar initial delay was detected by Baker & Crawford (1975) on the motor nerve terminal. This could be the result of a slow Na-dependent binding of ouabain to the Na-pump (cf. Baker & Crawford, 1975). In the presence of excess K (23.6 mM; 4 times normal) the resting outflow of [^{3}H]-NA did not increase but the action of ouabain was significantly inhibited (Figure 2a, Table 2). On the other hand, the initial delay before ouabain-evoked [^{3}H]-NA release was shortened (Figure 2a, Table 2). In another series of experiments excess Ca (7.5 mM) was used, and ouabain-evoked NA release was studied. Excess Ca did not affect the resting outflow of [^{3}H]-NA, but did significantly inhibit the transmitter-releasing action of ouabain (Figure 2b, Table 2) and prolonged the initial delay. When 23.6 mM K and 7.5 mM Ca were applied together 30 min before

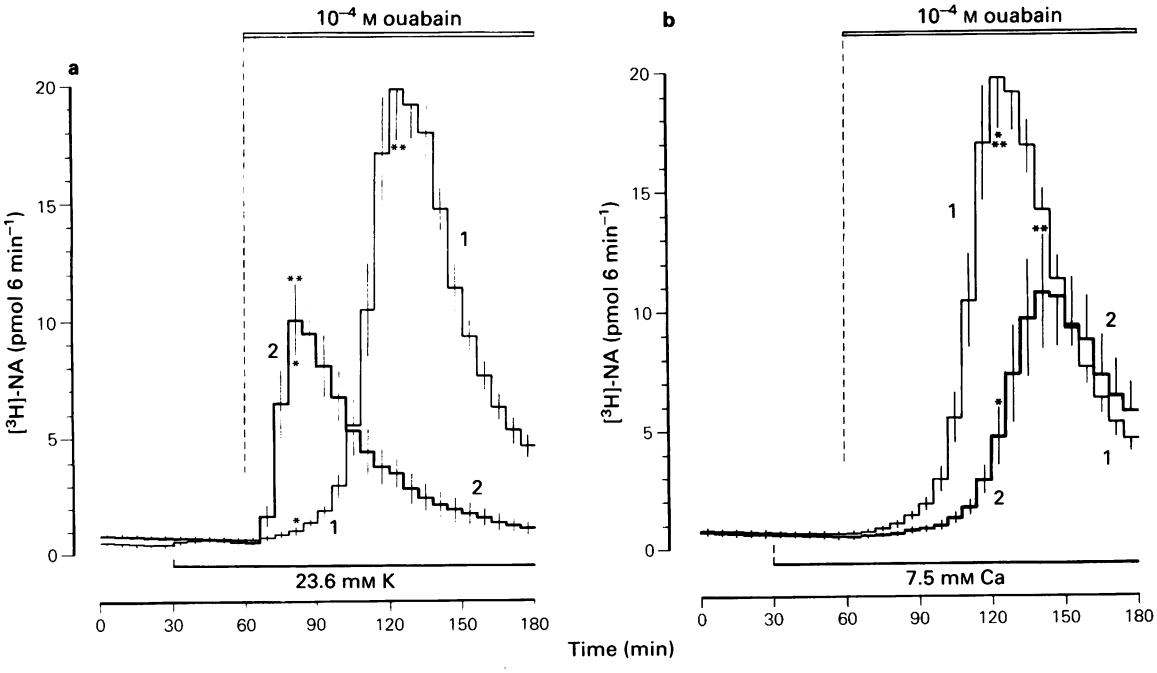

Figure 2 The [^{3}H]-noradrenaline ([^{3}H]-NA) releasing action of ouabain 10^{-4} M in the presence and absence of excess K (23.6 mM) and Ca (7.5 mM). The [^{3}H]-NA release expressed in pmol $6\,min^{-1}$ (ordinate scale) was plotted against the time (abscissa scale, min). (a) In normal Krebs solution ouabain 10^{-4} (curve No. 1) transiently increased the ^{3}H release after an initial delay ($n = 8$). Excess K (30 min preperfusion, curve No. 2) inhibited the NA releasing action of ouabain and shortened the initial delay ($n = 7$). Significant differences between values: $*P < 0.001$; $**P < 0.01$. (b) Ca 7.5 mM (30 min preperfusion) inhibited the ouabain-evoked release of NA (curve No. 2) and prolonged the initial delay ($n = 6$). Curve No. 1: ouabain alone for comparison. Significant differences between values: $*P < 0.001$; $**P < 0.05$.

Table 2 The effect of excess potassium (23.6 mM) and calcium (7.5 mM) on ouabain (10^{-4} M)-evoked [^{3}H]-noradrenaline release from the isolated main pulmonary artery of the rabbit

	Treatment	Δ pmol 6 min^{-1}*	*Peak release* (min)	*Significance* (*P*)	*Total amount of released [^{3}H]-NA* (pmol 120 min)
(1)	10^{-4} M ouabain	19.14 ± 2.13 (8) $P<0.001$	66		157.04 ± 12.33
(2)	23.6 mM K (30 min) + 10^{-4} M ouabain	9.55 ± 1.54 (7) $P<0.01$	24	2/1 $P<0.02$	75.33 ± 10.05
(3)	7.5 mM Ca (30 min) + 10^{-4} M ouabain	10.35 ± 2.34 (6) $P<0.02$	84	3/1 $P<0.05$	91.67 ± 23.69
(4)	23.6 mM K + 7.5 mM Ca (30 min) + 10^{-4} M ouabain	7.86 ± 0.23 (5) $P<0.001$	42	4/1 $P<0.01$	65.46 ± 5.34
(5)	23.6 mM K (30 min) + 7.5 mM Ca and 10^{-4} M ouabain together	19.08 ± 3.01 (7) $P<0.001$	36	5/2 $P<0.02$ 5/3 $P<0.05$ 5/4 $P<0.02$	145.81 ± 20.49
(6)	7.5 mM Ca (30 min) + 23.6 mM K and 10^{-4} M ouabain together	20.74 ± 3.17 (6) $P<0.001$	36	6/2 $P<0.01$ 6/3 $P<0.05$ 6/4 $P<0.01$	157.86 ± 23.22

*Ouabain-evoked peak release minus the last resting output obtained before treatment.
Means ± s.e.mean are given, number of experiments in parentheses.

ouabain application, the inhibition of the NA releasing effect of the cardiac glycoside did not increase further (Figure 3, Table 2), but the prolongation of the initial delay caused by excess Ca was shortened, and the peak release developed earlier (Table 2).

Neurotransmitter release in response to simultaneous application of ouabain and excess calcium after excess potassium preperfusion

An interesting and unexpected observation was made when ouabain (10^{-4} M) was applied together with excess Ca in excess K-containing solution. First, the preparation was preperfused for 30 min with 23.6 mM K, which again did not affect the outflow of NA. After 30 min, ouabain plus 7.5 mM Ca was introduced into the excess K-containing medium and this enhanced the NA release (Figure 4, Table 2). The rate of release did not differ significantly from the control; however, the initial delay was shortened and the peak release developed earlier (Table 2). The transmitter outflow did not change when excess Ca was added alone to the excess K-containing solution.

[^{3}H]-noradrenaline release in response to simultaneous application of ouabain and excess potassium after excess calcium preperfusion

A similar observation was made when excess Ca was used first, before the application of ouabain together with 23.6 mM K. By itself, 7.5 mM Ca did not change

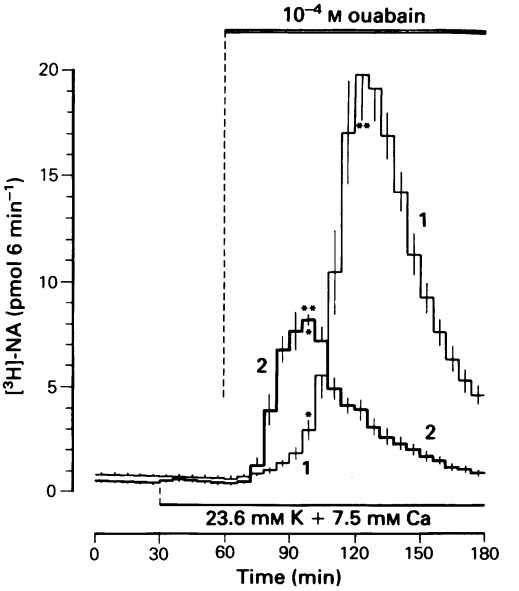

Figure 3 The inhibitory action of excess K (23.6 mM) and Ca (7.5 mM) on ouabain (10^{-4} M)- stimulated [^{3}H]-noradrenaline ([^{3}H]-NA) release (n = 5). Excess K and Ca were perfused for 30 min before ouabain application (curve No. 2). Curve No. 1: ouabain in normal Krebs solution for comparison. Note that excess K and Ca inhibited the neurotransmitter releasing action of ouabain and shortened the initial delay. Significant differences between values: *$P<0.001$; **$P<0.01$.

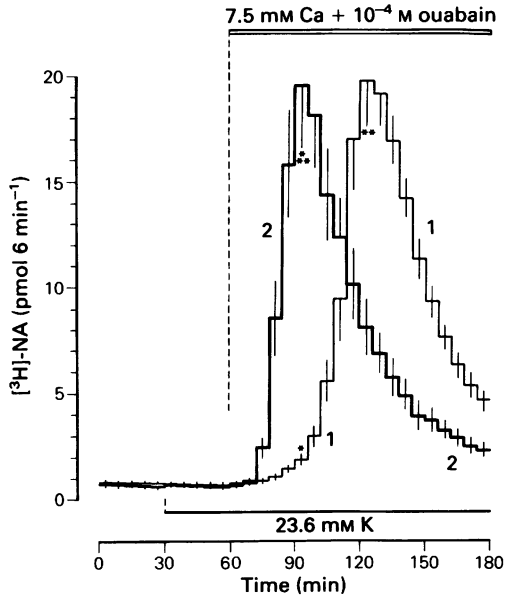

Figure 4 The lack of inhibitory action of excess K (23.6 mM) on ouabain (10^{-4} M)-evoked [^{3}H]-noradrenaline ([^{3}H]-NA) release: 23.6 mM K was perfused 30 min before ouabain plus 7.5 mM Ca application. Excess Ca antagonized the inhibition of ouabain-evoked NA release caused by excess K (curve No. 2), when it was applied together with ouabin ($n = 7$). Curve No. 1: ouabain for comparison. Differences between values: $*P < 0.001$; $**P > 0.9$. Note that the initial delay of ouabain-evoked NA release was shortened.

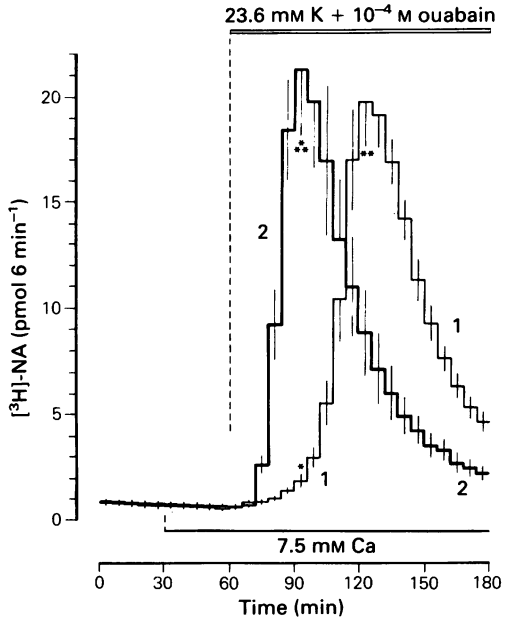

Figure 5 The lack of inhibitory action of excess Ca (7.5 mM) on ouabain (10^{-4} M)-stimulated neurotransmitter release. Excess Ca was applied 30 min before ouabain plus excess K (23.6 mM) perfusion. Excess K antagonized the inhibition caused by excess Ca of ouabain-stimulated NA release (curve No. 2), and shortened the initial delay ($n = 6$). Curve No. 1: ouabain alone in normal external medium. Differences between values: $*P < 0.001$; $**P > 0.6$.

the resting outflow of labelled neurotransmitter during 30 min preperfusion but on application of ouabain and excess K [^{3}H]-NA release was greatly increased (Figure 5, Table 2). The rate of NA release did not differ from the control and the initial delay was shortened (Figure 5, Table 2). The resting transmitter outflow was also not affected when excess K was applied alone after 30 min excess Ca preperfusion.

Discussion

Inhibition of the sodium-pump increased neurotransmitter release in the main pulmonary artery of the rabbit as in many other preparations (Banks, 1967; Birks & Cohen, 1968; Paton *et al.*, 1971; Vizi, 1972; 1977; Baker & Crawford, 1975; Bonaccorsi *et al.*, 1977; Nakazato *et al.*, 1978; Powis, 1983; Török *et al.*, 1984). In response to Na-pump inhibition the influx of Ca increases either as a result of depolarization or by activation of a reversed Na/Ca exchange (cf. Baker *et al.*, 1969; Baker, 1972; Baker & Crawford, 1975; Blaustein, 1974; Blaustein & Russell, 1975; Blaustein & Nelson, 1982; Mullins, 1977). During Na-pump inhibition the sodium gained inside can produce calcium release from internal stores (Baker & Crawford, 1975; Baker, 1976; Baker & Dipolo, 1984; Schoffelmeer & Mulder, 1983; Török *et al.*, 1984) which could be at least partly responsible for the neurotransmitter release observed. In the main pulmonary artery of the rabbit, K-removal prevented the initial delay of ouabain-evoked increase in neurotransmitter release (Figure 1a). Since the binding of ouabain to the Na-pump is Na-dependent (Baker, 1968; Baker & Manil, 1968; Baker & Willis, 1970; 1972; Baker & Crawford, 1975; Akera, 1977) and can be antagonized by external K (Schatzmann, 1953; Glynn, 1964; Garrahan & Glynn, 1967; Matsui & Schwartz, 1968; Baker & Willis, 1970; 1972; Lindenmayer & Schwartz, 1973; Schwartz *et al.*, 1975; Schwartz, 1976; Glynn & Karlish, 1975; Török *et al.*, 1984) this experimental finding confirms the well-known competition between Na and K for a common external activation site (Baker & Connelly, 1966; Baker & Willis, 1970; 1972; Lindenmayer & Schwartz, 1973; Schwartz *et al.*, 1975). The amount of NA

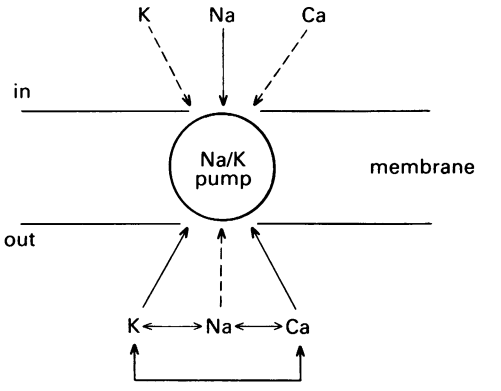

Figure 6 The Na/K exchange pump of cell membrane. At the external activation site of the Na-pump a Na-K, a theoretical Na-Ca and K-Ca competition is indicated: ←——, activation; ←– – –, inhibition.

released in response to ouabain in 'K-free' solution decreased (compare Figure 1a and Figure 2). This could be the consequence of a depolarization of the nerve terminal membrane in 'K-free' solution and/or an activation of the Na-pump by a rise in internal Na which had occurred and which partly counteracted the inhibitory action of ouabain. Furthermore there may be a partial saturation of the Na_i-Ca_o exchange mechanism by the elevated level of internal Na and a partial release of internally stored Ca by the Na previously gained. In 'K-free' solution, excess Ca (7.5 mM) inhibited NA release (Figure 1b). This could be the result of an activation of the Na-pump by Ca. It is known that Ca activates the neuronal Na,K-ATPase (Powis *et al.*, 1983), decreases internal Na activity already elevated by Na-pump inhibition (Deitmer & Ellis, 1978), inhibits ouabain binding to myocardial cells (Meldgaard *et al.*, 1981) and further delays the ouabain-evoked rise of the miniature endplate potential (Baker & Crawford, 1975). In the experiments of Deitmer & Ellis (1978), hyperpolarization developed in 'K-free' solution in response to excess Ca, a condition known to inhibit the neurotransmitter release (Vizi, 1978). It is unlikely that in response to excess Ca the activation of a Na_i-Ca_o exchange would be responsible for the inhibition of NA release observed, since the entry of Ca promotes transmitter release (Miledi, 1973). It is possible however, that Ca activates the K-efflux from the smooth muscle cells of the pulmonary artery (Ca_i-activated K-channels; Gárdos, 1958; cf. Meech, 1978; Marty, 1983; Peterson & Maruyama, 1984) leading to activation of the nerve terminal Na-pump. This possibility was excluded by using K-depleted tissues (that had been exposed for 5 h to 'K-free' solution), where excess Ca was also effective in inhibiting the [^{3}H]-NA release Török & Tóth, unpublished observation). Therefore it is plausible to suppose that the activation of the Na-pump by Ca leads to a re-establishment of the Na-gradient across the membranes of the nerve terminals and internal stores, resulting in Ca-loss from the cytoplasm and Ca-gain in the internal stores. On the other hand, it is not clear what sort of cation exchange is occurring on the Na-pump since the external medium does not contain K. It is unlikely that a ouabain-sensitive uncoupled Na-efflux (Baker, 1964; Beaugé & Ortiz, 1972; cf. Glynn & Karlish, 1975) would be responsible for the re-established Na-gradient since the external medium contained Na. The inhibition of 'K-free' stimulated NA release by excess Ca was antagonized by ouabain (Figure 1b). Since the action of ouabain is Na-dependent in the rabbit pulmonary artery (Török *et al.*, 1984) the inhibition of 'K-free' stimulated NA release by Ca and its antagonism by ouabain suggests a Na-Ca competition at the external activation site of the Na-pump, originally suggested by Repke (1963), similar to that of Na and K. Excess Ca also inhibited the ouabain-evoked [^{3}H]-NA release and increased the initial delay (Figure 2b). In normal external ionic environments the initial delay of ouabain-evoked neurotransmitter release is presumably the consequence of a slow Na-dependent binding of ouabain to the Na-pump (Baker & Crawford, 1975; Török *et al.*, 1984). In the presence of excess Ca the ouabain-evoked transmitter release was inhibited by about 50% (Table 2), a value which did not increase further when both excess Ca and K were applied together 30 min before ouabain perfusion (see Figures 2b and 3; Table 2). On the other hand, the initial delay was shortened in excess Ca and K-containing solution. An unexpected observation was made in the rabbit pulmonary artery, namely that excess K failed to antagonize the NA-releasing action of ouabain when after 30 min preperfusion with excess K containing solution, the cardiac glycoside was applied together with excess Ca (Figure 4). The rate of release did not differ significantly from the control and the initial delay was markedly shortened (Table 2). The converse of these results proved to be true also, i.e. excess Ca was not able to inhibit the NA releasing action of ouabain when ouabain was applied together with excess K (Figure 5, Table 2) after 30 min exposure to 7.5 mM Ca. Under these conditions the initial delay was also shortened and the rate of release was even bigger than the control. The interpretation of these results remains obscure. A theoretical explanation could be a sort of K and Ca competition at the external activation site of the Na-pump (Figure 6), with Ca antagonizing the inhibition by K of the Na-dependent binding of ouabain to the Na-pump; thus K exerts a similar antagonistic action on activation of the Na-pump by Ca. It must be mentioned that Schwartz (1976) has also suggested a relationship between K and Ca at the K-activation site.

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