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RELEASE OF PURINES AND NORADRENALINE BY OUABAIN AND POTASSIUM CHLORIDE FROM VASCULAR ADRENERGIC NERVES

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1 Release of [^{3}H]-noradrenaline and ^{3}H-purine by ouabain (10^{-4} M) or high KCl (50 mM) was investigated in the superfused rabbit pulmonary arterial segment preincubated with [^{3}H]-noradrenaline or [^{3}H]-adenosine.

2 Ouabain elicited a delayed large contraction and a parallel [^{3}H]-noradrenaline efflux. These were substantially inhibited by Ca^{2+}-free medium or preincubation with 6-hydroxydopamine (30 μg/ml, 30 min).

3 Ouabain caused an ^{3}H-purine efflux which was slower than the [^{3}H]-noradrenaline efflux. This was inhibited by 6-hydroxydopamine and in part by phentolamine (3×10^{-6} M), indicating both neuronal and extraneuronal origins of purines.

4 In contrast to ouabain, high KCl appeared to induce predominantly purine efflux, which was phentolamine-insensitive and 6-hydroxydopamine-sensitive, indicative of neuronal origin.

5 It is suggested that the purine efflux evoked by ouabain and high KCl may originate from different neuronal vesicles.

Introduction

Membrane depolarization produced by high concentrations of KCl (Thoa, Wooten, Axelrod & Kopin, 1975; Garcia, Kirpekar & Pasacual, 1978) and veratridine (Thoa *et al.*, 1975; Minchin, 1980) is capable of facilitating the noradrenaline release from adrenergic nerves. Procedures that inhibit Na^+,K^+-ATPase such as ouabain and K^+-removal also enhance the release of noradrenaline in various tissues (Paton, 1973; Garcia & Kirpekar, 1973; Ozawa & Katsuragi, 1974; Bonaccori, Hermsmyer, Smith & Bohr, 1977; Katsuragi, Fukushi & Suzuki, 1978), probably via the Na^+–Ca^{2+} exchange system (Gillis & Quest, 1980). When noradrenaline is liberated by a chemical or electrical stimulus, ATP and other substances contained in the nerve vesicles appear to be released together with the neurotransmitter (Lagercrantz, 1976). It is uncertain whether purines are released from extraneuronal or postsynaptic sites as well as neuronal sites (Su, 1975; Fredholm & Hedqvist, 1978; Luchelli-Fortis, Fredholm & Langer, 1979; Katsuragi & Su, 1980; 1981; 1982; Fedan, Hagaboom, O'Donnell, Colby & Westfall, 1981). At high concentrations, KCl evokes a Ca^{2+}-dependent purine efflux from various tissues, e.g., guinea-pig cerebral cortical synaptosomes (White, 1978; Potter & White, 1980) and rabbit vascular adrenergic nerves (Katsuragi & Su, 1980; 1981; 1982). Hollins & Stone (1980) found that ouabain (10^{-4} M) as well as KCl elicited a Ca^{2+}-dependent efflux of ^{3}H-purines from rat cerebral cortex slices. The present study was, therefore, designed to compare the releasing effects of ouabain and high KCl on noradrenaline and purines with particular reference to their origins.

Methods

Male adult rabbits, weighing 2.0–2.5 kg, were anaesthetized with pentobarbitone (40 mg/kg, i.v.) and exsanguinated. The main pulmonary artery was dissected out and cut into a spiral strip. A segment of this was placed between two platinum electrodes and one end was anchored to a stationary support and the other connected to a Grass (F.03) strain gauge. It was immersed at 37.5°C for 30 min in Krebs-bicarbonate solution of the following composition (mM): NaCl 122, $NaHCO_3$ 25.6, KCl 5.2, $CaCl_2$ 2.4, $MgSO_4.7H_2O$ 1.2, glucose 11, ascorbic acid 0.1 and Na_2EDTA 0.03. After incubation with [^{3}H]-adenosine (10^{-7} M) for 2 h or [^{3}H]-noradrenaline (10^{-7} M) for 1.5 h, the strip preparation was rinsed.

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Superfusion was carried out at 3 ml/min with Krebs solution (37.5°C). The superfusate was collected every 2 min and its tritium activity assayed by liquid scintillation spectrometry. Ouabain was added to the superfusate for the 24th to the 48th fractions. In other preparations high KCl (50 mM) was added for 2 min at the 32nd fraction. The corresponding contraction was recorded on a polygraph. Phentolamine was introduced at the 21st fraction before the administration of ouabain or KCl. *In vitro* chemical denervation of the pulmonary arterial segment was achieved by 6-hydroxydopamine (30 μg/ml) added to Krebs solution containing ascorbic acid (1 mg/ml) 30 min before incubation with tritium. Success of the denervation was assessed by the disappearance of the contractile response to electrical stimulation (10 Hz, 0.3 ms, supramaximal voltage) but not to KCl or (−)-adrenaline (Katsuragi & Su, 1980).

As a measure of the total ouabain-induced ^{3}H-efflux, the following calculation was used, assuming efflux in the 6-hydroxydopamine (6-OHDA)-treated preparation to be equivalent to the basal efflux:

$$\frac{\text{Total efflux (fractions 24–48)} - \text{total efflux, 6-OHDA treated (fractions 24–48)}}{\text{Control efflux (fraction 23)}} \times 100$$

Similarly, the total KCl-evoked efflux was expressed as the total efflux from fractions 32–37 minus the total efflux from 6-OHDA-treated preparations, relative to the control efflux (fraction 31). In fact, in both cases, ^{3}H effluxes from the preparation treated with 6-OHDA were approximately the same as the basal effluxes although we did not illustrate this point in the present figures. Ouabain- or KCl-induced purine efflux, which is phentolamine-resistant and 6-OHDA-sensitive, is assumed to be of presynaptic origin. Total ^{3}H activity in the present study was used as the measure of [^{3}H]-noradrenaline or ^{3}H-purine efflux; the proportion of the total appearing as ^{3}H-metabolites was not measured. The [^{3}H]-noradrenaline uptake was calculated as the tissue-medium ratio after exposure to 0.1 μM [^{3}H]-noradrenaline for 1.5 h as described previously (Katsuragi & Su, 1980; 1982). Student's *t* test was used for analysis of significance of difference between two means. A probability less than 0.05 was accepted as significant.

Drugs used were ouabain (E. Merck); phentolamine hydrochloride (Ciba-Geigy); 6-hydroxydopamine hydrobromide (Sigma); [^{3}H]-adenosine (specific activity 36.2 Ci/mmol, New England Nuclear) and [^{3}H]-noradrenaline (specific activity 50 Ci/mmol, Amersham).

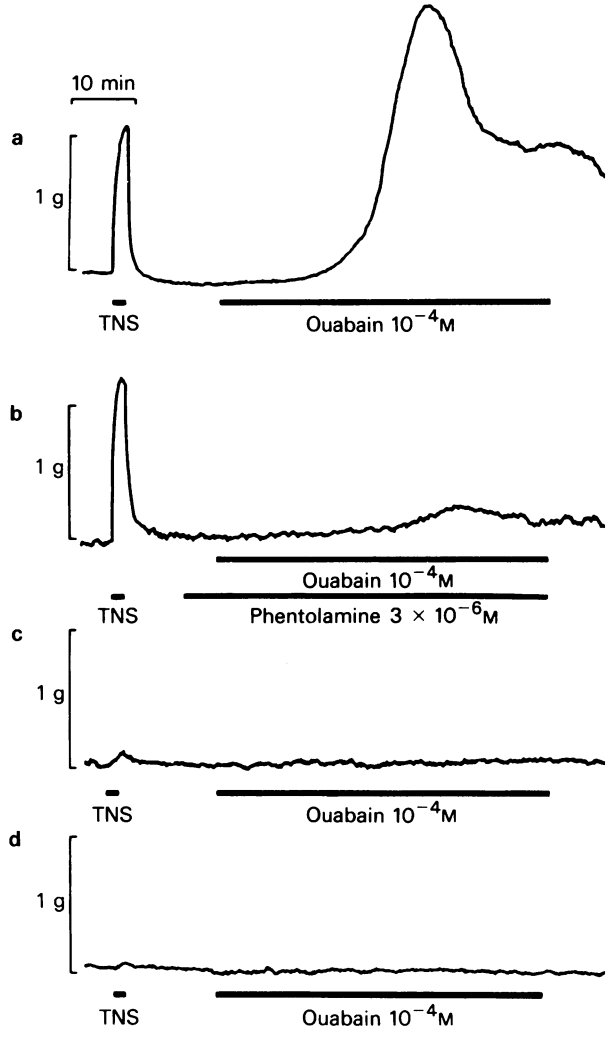

Figure 1 Typical examples of influence of phentolamine and 6-hydroxydopamine on the ouabain-induced contraction of the pulmonary artery: (a) ouabain alone; (b) phentolamine plus ouabain; (c) 6-hydroxy-dopamine (30 μg/ml, 30 min) plus ouabain; (d) Ca^{2+}-free medium plus ouabain. TNS: transmural nerve stimulation. These traces came from four different preparations.

Results

Ouabain-induced contraction

When 10^{-4} M ouabain was introduced into the superfusate, a contraction of the tissue occurred with a latency of about 10 min. This reached peak tension in 28 to 30 min, then declined rapidly despite the continued presence of ouabain. The ouabain-induced contraction was markedly attenuated by superfusion

with 3×10^{-6} M phentolamine and Ca^{2+}-free medium 6 min and 48 min before administration of ouabain, respectively. Further, this contraction did not occur in the vascular segment pretreated with 6-OHDA for 30 min.

Typical examples of each treatment from three experiments are illustrated in Figure 1.

Ouabain-induced [^{3}H]-noradrenaline efflux

Exposure to 10^{-4} M ouabain for 48 min elicited an efflux of [^{3}H]-noradrenaline from the rabbit pulmonary artery. This efflux curve is in good agreement with the corresponding contraction. The peak efflux was recorded on the 38th fraction. The ouabain-induced [^{3}H]-noradrenaline efflux was prevented by Ca^{2+} removal from the superfusate 48 min before the introduction of ouabain. The ^{3}H efflux evoked by ouabain was abolished by *in vitro* pretreatment with 6-OHDA 30 min before incubation with [^{3}H]-noradrenaline. These results are summarized in Figure 2. In additional experiments, the tissue-medium ratio indicating [^{3}H]-noradrenaline uptake into the vascular segment was 5.91 ± 0.65 ($n = 11$) in normal preparations and 0.85 ± 0.15 ($n = 4$) in the 6-OHDA-treated preparations.

Ouabain-induced ^{3}H-purine efflux

Superfusion with 10^{-4} M ouabain evoked the efflux of ^{3}H-purine from the vascular segment. This ^{3}H-efflux curve rose approximately linearly from the 33rd to the 48th fraction. The high efflux level at the 48th fraction was maintained for several additional fractions even after removal of ouabain from the superfusate. This evoked efflux was markedly decreased by pretreatment with 6-OHDA and was moderately reduced by the presuperfusion with 3×10^{-6} M phentolamine during the 21st to the 48th fractions (Figure 3). Phentolamine was used to prevent purine release by noradrenaline, which had already been released from nerve terminals and was acting on α-receptors.

Relative effluxes of [^{3}H]-noradrenaline and ^{3}H-purines

The 6-OHDA-sensitive effluxes of [^{3}H]-noradrenaline and ^{3}H-purine were enhanced by exposure to 10^{-4} M ouabain for 48 min by 834.2 and 349.2%, respectively. These figures are a measure of total evoked release calculated as described in the Methods section. This purine release was decreased to 140.5% by 3×10^{-6} M phentolamine. These effluxes were enhanced by contact with 50 mM KCl for 2 min by 42.4 and 254.7%, respectively. This high KCl-induced ^{3}H-purine efflux, which was sensitive to 6-OHDA, was practically unaltered by phentolamine, suggesting predominantly presynaptic release and confirming our earlier finding (Katsuragi & Su, 1980). Increase in [^{3}H]-noradrenaline efflux evoked by 10^{-4} M ouabain was approximately 6 fold greater than presynaptic ^{3}H-purine efflux evoked by the drug. By contrast, increase in the ^{3}H-purine efflux following 50 mM KCl was approximately 6 fold [^{3}H]-noradrenaline efflux evoked by KCl (Table 1).

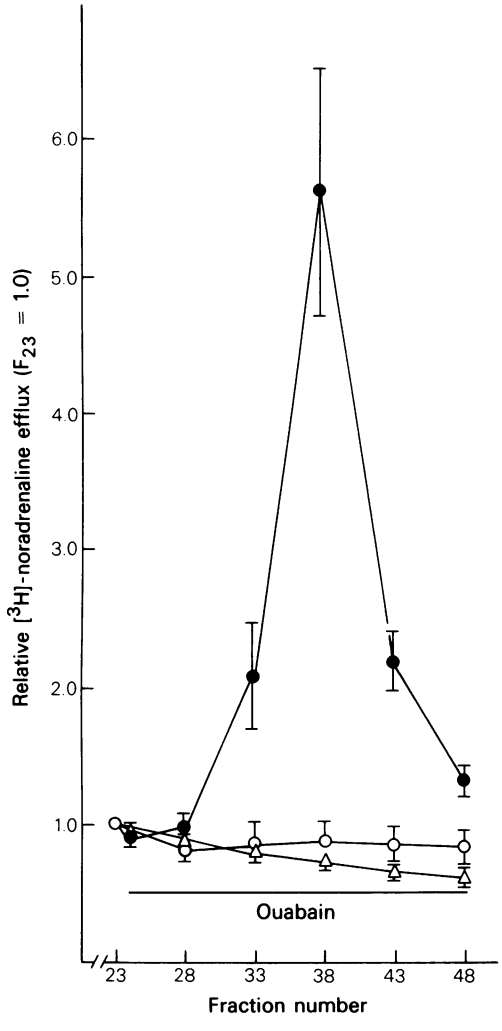

Figure 2 Influence of Ca^{2+}-free medium and 6-hydroxydopamine on the ouabain-induced noradrenaline release from the pulmonary artery. (●) 10^{-4} M ouabain alone, introduced at the 24th fraction; (○) Ca^{2+}-free medium plus ouabain; (△) 6-hydroxydopamine (30 μg/ml, 30 min) plus ouabain. Each point denotes relative efflux and shows mean of 4 experiments (vertical bars show s.e.mean), taking d/min of the 23rd fraction as 1.0.

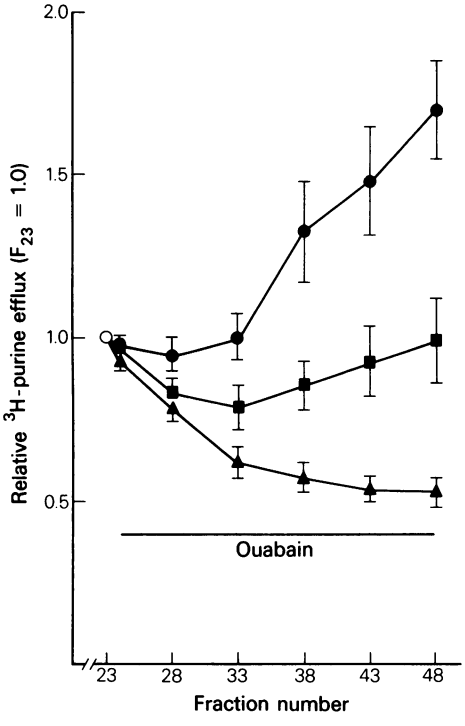

Figure 3 Influence of phentolamine and 6-hydroxydopamine on the ouabain-induced purine efflux from the pulmonary artery. (●) 10^{-4} M ouabain; (■) 3×10^{-6} M phentolamine plus ouabain; (▲) 6-hydroxydopamine (30 μg/ml, 30 min) plus ouabain. For other details, see Figure 2.

Discussion

In the present study, ouabain (10^{-4} M) caused a contractile response of the rabbit pulmonary artery which was inhibited by an α-blocking agent, phentolamine, by Ca^{2+}-removal from the superfusate and by adrenergic denervation with 6-OHDA. In addition, the time course of the contraction was in good accord with the efflux of [^{3}H]-noradrenaline, both peaking at around the 38th fraction. Like the contraction, the [^{3}H]-noradrenaline efflux was prevented by pretreatment with 6-OHDA or Ca^{2+}-removal from the superfusate. Absence of [^{3}H]-noradrenaline uptake as a result of pretreatment with 6-OHDA was confirmed in this vascular segment as expected. Ouabain, therefore, elicited a Ca^{2-}-dependent noradrenaline release from the pulmonary arterial adrenergic nerves which was the cause of the contractile response.

In contrast to the [^{3}H]-noradrenaline efflux, the curve for ^{3}H-purine efflux induced by ouabain lapsed behind the curve for tension in a manner that was difficult to explain. The maximum efflux did not occur until 20 min after that of [^{3}H]-noradrenaline efflux or contraction. The time course of ^{3}H-purine efflux was nearly identical to that from the rat cerebral cortex slice described by Hollins & Stone (1980).

As previously reported (Katsuragi & Su, 1980), the KCl-induced release of both ^{3}H-purine and [^{3}H]-noradrenaline were abolished by denervation with 6-OHDA. There are two representative dense cored vesicles, large granular (600–1200 Å) and small granular (300–600 Å) vesicles, in varicosities of adrenergic nerves (Lagercrantz, 1976; Fried, 1980; Burnstock, 1980). Noradrenaline is thought to coexist with ATP in various binding ratios in the adrenergic nerve vesicles. As reviewed by Fried (1980), the noradrenaline/ATP ratios in the purified large and small granular vesicles are 7.5 to 12.0:1 and 20.0 to 60.0:1, respectively. Segmentation of splenic nerves gave noradrenaline/ATP ratios of 5.7:1 and 7.4:1 in a proximal plus medial portion and a distal portion, respectively (Lagercrantz, 1976). It is possible that ouabain selectively affects the noradrenaline-dominant vesicular component, e.g., small granular vesicles. By contrast, high KCl may elicit release primarily from the large granular vesicles. [^{3}H]-noradrenaline efflux, unlike ^{3}H-purine efflux, was little increased by KCl. Release from different vesicles might in part account for the relatively greater amount of ATP released by KCl. Further-

Table 1 Comparison between [^{3}H]-noradrenaline and ^{3}H-purine efflux evoked by ouabain or high KCl

	[^{3}H]-noradrenaline (% increase of ^{3}H efflux)	*^{3}H-purines*
Ouabain 10^{-4} M	834.2 ± 142.5	349.2 ± 56.7 (presynaptic 140.5 ± 30.2)[a]
KCl 50 mM	42.4 ± 9.5	254.7 ± 55.5 (presynaptic 252.2 ± 50.3)[a]

Values are the means from four or five experiments ± s.e.mean.
[a]Presynaptic represents phentolamine-resistant and 6-hydroxydopamine-sensitive ^{3}H-efflux.

more, whereas KCl-induced ^{3}H-purine efflux is insensitive to phentolamine, the ouabain-induced efflux was roughly halved by this α-receptor blocking agent. This suggests that the ouabain-induced purine efflux may arise in part extraneuronally and be mediated by noradrenaline liberated from the adrenergic nerves. In addition to being phentolamine-insensitive, the ^{3}H-purine efflux caused by high KCl was not at all affected by contractile activity (Katsuragi & Su, 1982). These facts support our previous understanding (Katsuragi & Su, 1980) that a depolarization evoked by KCl enhances the presynaptic release of purine from vascular adrenergic nerves.

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