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FACILITATION BY CLONIDINE OF PURINE RELEASE INDUCED BY HIGH KCl FROM THE RABBIT PULMONARY ARTERY

TAKESHI KATSURAGI[1] & CHE SU

Department of Pharmacology, Southern Illinois University, School of Medicine, Springfield, Illinois 62708, U.S.A.

1 The effect of clonidine on the ^{3}H-purine release evoked by KCl or (−)-adrenaline was assessed in the superfused helical strip of the rabbit pulmonary artery pretreated with [^{3}H]-adenosine.

2 Clonidine (3×10^{-5} M to 10^{-4} M) significantly enhanced the ^{3}H-purine efflux evoked by 50 mM KCl but not by 3×10^{-6} M (−)-adrenaline.

3 This facilitatory effect of clonidine on the KCl-induced purine release was unaltered by phentolamine 3×10^{-6} M. It was absent in arterial segments denervated with 6-hydroxydopamine 30 μg/ml.

4 A sustained contractile response was evoked by clonidine 3×10^{-5} M without an increase in the ^{3}H-purine efflux. This was significantly reduced by phentolamine 3×10^{-6} M, but not by yohimbine 10^{-5} M or by denervation with 6-hydroxydopamine.

5 The uptake of [^{3}H]-adenosine into the segments was not inhibited by clonidine 3×10^{-5} M.

6 It is suggested that the facilitation by clonidine of the KCl-induced purine release is due to prevention of presynaptic autoinhibition of purine release from adrenergic nerves, by an anti-adenosine action of the drug.

Introduction

Adenosine or adenosine 5′-triphosphate (ATP) applied exogenously inhibits noradrenaline release from the adrenergic nerve endings. Their inhibitory effect is antagonized by theophylline (Hedqvist & Fredholm, 1976; Clanachan, Johns & Paton, 1977; Verhaeghe, Vanhoutte & Shepherd, 1977), a P_1-purinoceptor blocker (Burnstock, 1978), probably by blocking the presynaptic receptor. It has therefore been postulated that adenosine or ATP may play a role of a neuromodulator and co-neurotransmitter, together with noradrenaline (Su, 1975; 1978; Burnstock, 1978; Fredholm & Hedqvist, 1980; Feden, Hogaboom, O'Donnell, Colby & Westfall, 1981).

To elucidate the precise mechanism of neuronal release of purine compounds has been difficult, due in part to their neuronal and extraneuronal distribution. We have found that depolarizing concentrations of KCl evoked a Ca^{2+}-dependent presynaptic purine release in the adrenergically innervated rabbit pulmonary artery, whereas (−)-adrenaline caused the release postsynaptically even after denervation with 6-hydroxydopamine (Katsuragi & Su, 1980). The purine release evoked by high KCl but not by adrenaline appears to be facilitated by 10^{-6} to 10^{-5} M theophylline, probably through a blockade of presynaptic autoinhibition (Katsuragi & Su, 1981).

Stone & Taylor (1978a,b) have demonstrated that iontophoretically applied clonidine antagonized the depressant effects of purine compounds without affecting responses to noradrenaline, 5-hydroxytryptamine or γ-aminobutyric acid on the firing rate of rat cerebral cortex neurones. They concluded that clonidine, as well as other 2-substituted imidazoline derivatives such as phentolamine, had an anti-adenosine property. The present study assessed the anti-adenosine activity of clonidine by measurement of its ability to enhance the KCl-induced purine release from the pulmonary arterial segment.

Methods

Procedures

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 and cut into a spiral strip, a segment of which was placed between two platinum electrodes with one end of the strip 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

[1]Present address: Department of Pharmacology, Fukuoka University School of Medicine, 34 Nanakuma, Fukuoka 814, Japan.

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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 disodium edetate (Na_2EDTA) 0.03. After incubation with [^{3}H]-adenosine 10^{-7} M for 2 h, the strip preparation was rinsed. 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 in a Mark III Liquid Scintillation Counter. KCl or (−)-adrenaline was added for 2 min at the 32nd and 48th fractions, whereas a test drug was introduced for 16 min during the 40th to 47th fractions. The corresponding contraction was recorded on a polygraph. To test the effect of drugs such as clonidine on the uptake of [^{3}H]-adenosine into this tissue, they were introduced into the incubation medium 30 min before [^{3}H]-adenosine. The tissue was blotted on filter paper, weighed and digested with Soluene (Packard Instrument Company, Inc., Downers Grove, IL) and its activity was assayed. A small amount of radioactive incubation medium was also assayed for tritium activity.

In vitro denervation of the pulmonary arterial segment was achieved by 30 min exposure before incubation to 6-hydroxydopamine 30 μg/ml in Krebs solution containing ascorbic acid (1 mg/ml). Specific denervation was confirmed by disappearance of the contraction response to electrical transmural nerve stimulation (10 Hz, 0.3 ms, supramaximal voltage) but not to KCl or (−)-adrenaline (Katsuragi & Su, 1980).

Evaluations

The net increases in tritium efflux during the 10 min after addition of KCl or (−)-adrenaline in the 32nd and 48th fractions were calculated as percentage increases over the tritium efflux in the 31st and 47th fractions. They were designated S_1 and S_2, respectively. The inhibition of tritium uptake by drugs was expressed as percentage change in the tissue-medium ratio, i.e., the ratio between ^{3}H activity in the tissue (per g) and that in the medium (per ml) immediately after [^{3}H]-adenosine incubation for 2 h. Student's *t* test was used for analysis of significance of difference between two means. A probability less than 0.05 was considered significant.

Drugs

Drugs used were (−)-adrenaline (Calbiochem-Behring); phentolamine hydrochloride (Ciba); yohimbine hydrochloride (Sigma); clonidine hydrochloride (Boehringer Ingelheim); dipyridamole (Ciba-Geigy) and [^{3}H]-adenosine (specific activity 36.2 Ci/mmol, New England Nuclear).

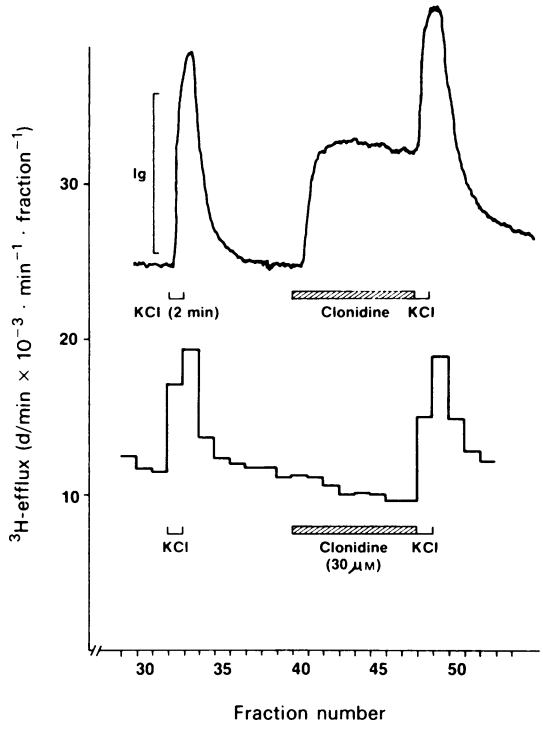

Figure 1 Typical example of the effect of clonidine 3×10^{-5} M on the isometric contraction (above) and ^{3}H-purine efflux (below) induced by KCl 50 mM in the rabbit pulmonary artery.

Results

Effects of clonidine and phentolamine on the high KCl- or adrenaline-induced release of ^{3}H-purine

High KCl and (−)-adrenaline were employed as pre- and postsynaptic purine releasers, respectively, as described earlier (Katsuragi & Su, 1980). KCl 50 mM or (−)-adrenaline 3×10^{-6} M greatly enhanced the net efflux of ^{3}H-purines from the arterial segment associated with a contractile response (Figure 1). When clonidine (10^{-5} to 10^{-4} M) was introduced into the superfusate, a contraction of the tissue was elicited and maintained during its presence. Nevertheless, the basal tritium efflux from the segment was not increased indicating that the mode of action of clonidine differed from that of KCl or (−)-adrenaline.

The KCl-induced ^{3}H-purine release was facilitated by 3×10^{-5} to 10^{-4} M clonidine in a concentration-related manner, while this release was unaffected by phentolamine 3×10^{-6} M (Figure 2). By contrast, the

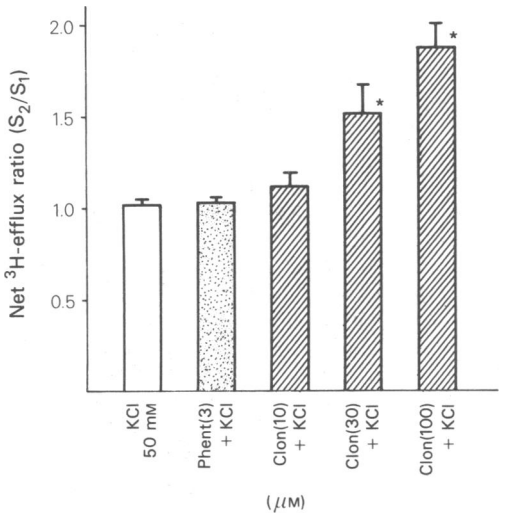

Figure 2 Effect of clonidine (Clon) or phentolamine (Phent) on the KCl (50 mM)-induced ^{3}H-purine release from the rabbit pulmonary artery. Mean values from 4–6 experiments are shown. Vertical lines indicate s.e.mean.
*$P<0.05$; **$P<0.001$, compared with KCl alone.

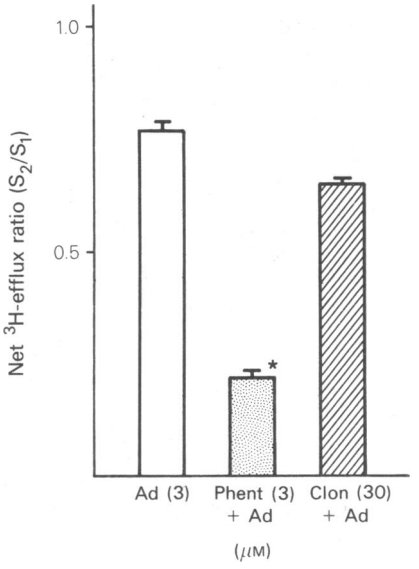

Figure 3 Effect of clonidine (Clon) or phentolamine (Phent) on the (−)-adrenaline (Ad, 3×10^{-6} M)-induced ^{3}H-purine release from the rabbit pulmonary artery. Mean of 4 experiments; vertical lines show s.e.mean.
*$P<0.001$ compared with adrenaline alone.

purine release evoked by (−)-adrenaline was decreased somewhat but not significantly by 3×10^{-5} M clonidine. A considerable reduction in adrenaline-induced purine release was caused by phentolamine 3×10^{-6} M (Figure 3).

Effects of phentolamine and denervation on the facilitation of ^{3}H-purine release by clonidine

Clonidine is known as a presynaptic α_2-adrenoceptor agonist (Langer, 1977; Starke, 1977). The significance of this activity in the facilitation of the KCl-induced purine release was, therefore, assessed. The enhancement by clonidine of the KCl-induced purine release was unaffected by the presence of phentolamine 3×10^{-6} M. Denervation considerably diminished the KCl-induced purine releases at S_1 and S_2, and lowered the S_2/S_1 ratio by approximately 40%. The KCl-induced purine release was not increased by clonidine 3×10^{-5} M following denervation. These results are shown in Table 1.

Table 1 Effects of denervation and phentolamine on facilitation by clonidine of KCl-induced ^{3}H-purine release in the pulmonary artery

Treatment (μM)	*S_2/S_1 ratio*
50mM KCl	1.02 ± 0.03 (6)
Clonidine (30) + KCl	1.52 ± 0.16 (6)
Phentolamine (3) + KCl	1.03 ± 0.03 (4)
Phentolamine (3) + clonidine (30) + KCl	1.49 ± 0.07 (4)
6-Hydroxydopamine + KCl	0.60 ± 0.03 (3)
6-Hydroxydopamine + clonidine (30) + KCl	0.69 ± 0.07 (3)

Values are mean ± s.e. (*n*).

Effects of drugs and denervation on the contraction evoked by clonidine

The sustained contractile response of the pulmonary arterial strip evoked by exposure to clonidine (3×10^{-6} M) was significantly reduced by phentolamine (3×10^{-6} M) but not by yohimbine (10^{-5} M) (an α-adrenoceptor blocking agent), introduced 4 min before clonidine. The clonidine-induced contraction did not diminish significantly following denervation with 6-hydroxydopamine. These results are summarized in Table 2.

Effect of clonidine on uptake of [^{3}H]-adenosine

Tritiated adenosine uptake into the pulmonary arterial segment was not significantly inhibited by clonidine (3×10^{-5} M), to 89.9 ± 11.1% ($n = 8$) of the control. However, it was strongly suppressed by dipyridamole 10^{-6} M known as an adenosine uptake inhibitor, to 16.6 ± 3.3% ($n = 5$, $P<0.001$).

Table 2 Effects of drugs and denervation on the contraction of the pulmonary artery evoked by clonidine

Treatment (μM)	*Tension* (g)
Clonidine (30)	0.98 ± 0.11 (6)
Phentolamine (3) + clonidine	0.57 ± 0.12* (6)
Yohimbine (10) + clonidine	0.86 ± 0.05 (5)
6-Hydroxydopamine + clonidine	0.88 ± 0.05 (4)

Values are mean ± s.e. (*n*).
*$P < 0.05$ compared with clonidine alone.

Discussion

Clonidine has been used clinically as a unique antihypertensive agent that stimulates α-adrenoceptors in the central nervous system (Van Zweiten, 1973; Haeusler, 1975). This drug also exhibits a variety of peripheral interactions with catecholamines, e.g. α_1- or α_2-adrenoceptor stimulation (Starke & Altmann, 1973; Langer, 1977), α_1-adrenoceptor blockade (Coupar & Kirby, 1972; Eltze, 1979; Ress, Field, Lockley & Fregly, 1979) and inhibition of extraneuronal amine uptake (Salt, 1972). In addition, Stone & Taylor (1978a,b) have postulated that clonidine may be an anti-adenosine agent because of its reversal of a central neuronal inhibitory response to adenosine.

In the present study, clonidine itself has been shown to elicit a sizable contraction of the pulmonary artery. This contraction was moderately reduced by phentolamine (3×10^{-6} M), but not by yohimbine 10^{-5} M or pretreatment with 6-hydroxydopamine. It appears likely that the contractile response is in part mediated by the postsynaptic α_1-adrenoceptor (Hodge & Robinson, 1972; Ress *et al.*, 1979). On the other hand, if a large portion of the contractile response to clonidine is attributable to the postsynaptic α_1-adrenoceptor stimulation, it should be accompanied by purine release as with adrenaline. However, we did not observe an appreciable ^{3}H-purine release by clonidine even at 10^{-4} M. Hence, there is little relationship between the contraction and purine release by clonidine. The phentolamine-resistant component of the clonidine-induced contraction of this vascular tissue cannot yet be explained.

As described earlier (Katsuragi & Su, 1981), theophylline (10^{-5} to 10^{-4} M) augmented the ^{3}H-purine release evoked by KCl, but not by (−)-adrenaline from the rabbit pulmonary arterial preparation. Clonidine mimicked the effect of this anti-adenosine agent as shown in the present study. The facilitation by clonidine of the KCl-induced purine release was abolished by denervation with 6-hydroxydopamine but not by phentolamine, an α-adrenoceptor blocking agent. Thus, the facilitation does not appear attributable to pre- or postsynaptic α-receptor stimulation by clonidine. As clonidine did not inhibit the uptake of [^{3}H]-adenosine by the pulmonary arterial segment, the facilitation is probably not due to prevention of re-uptake of liberated purines.

From these observations, it is suggested that the facilitatory effect of clonidine on the depolarization-induced purine release from adrenergic nerves results from antagonism of presynaptic inhibition (autoinhibition) of release by purines themselves. This possibly involves anti-adenosine action of the drug proposed by Stone & Taylor (1978a,b) on presynaptic adenosine receptors.

It has been postulated that membrane depolarization of the postsynaptic effector cells and/or local disparity in energy balance is responsible for release of adenosine compounds during nerve activity; vasoconstriction is deemed the crucial factor in several perfused tissues (Fredholm & Hedqvist, 1980). We observed earlier that after denervation of the rabbit pulmonary artery, high KCl effectively elicited contraction, presumably by smooth muscle membrane depolarization, but caused much smaller purine release (Katsuragi & Su, 1980). The present work has demonstrated arterial contraction by clonidine without appreciable purine release. It appears, therefore, that smooth muscle depolarization does not effectively mediate the KCl-evoked purine release and contraction is not causally related to purine release. On the other hand, in view of the adrenaline-induced release which is blocked by phentolamine, the α-adrenoceptor agonist-receptor interaction seems to lead to purine release from vascular smooth muscle.

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