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THE EFFECTS OF ADENINE NUCLEOTIDES ON CUTANEOUS AFFERENT NERVE ACTIVITY

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1 The activity produced by the adenine nucleotides adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) and by potassium, acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and bradykinin when applied to an exposed blister base on the ear of anaesthetized rabbits or intra-arterially to anaesthetized cats was investigated in multiple strands dissected from the auricular-temporal and saphenous nerves of rabbits and cats, respectively.

2 In the rabbit preparation potassium and the adenine nucleotides produced activity in the nerve fibres. The effects of these substances were produced in comparable dose ranges; threshold effects being produced by potassium at a concentration of 13 mM and by ADP at a concentration of 4 mM. ACh, 5-HT and bradykinin were inactive at similar or higher concentrations.

3 In the cat preparation all the substances tested produced activity in the nerve fibres. The adenine nucleotides were comparatively less potent than ACh, 5-HT or bradykinin, but had greater potency than potassium.

4 It was concluded that the adenine nucleotides do possess effects on afferent nerve terminals or fibres and thus resemble other known algogenic substances such as potassium, ACh, 5-HT and bradykinin.

Introduction

When potassium, acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and bradykinin are applied to a blister base on human forearm pain is produced (Keele & Armstrong, 1964). This property is shared by adenosine compounds (Bleehen, Hobbiger & Keele, 1976; Bleehen & Keele, 1977). Analysis of their activity at this sensory site showed that adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) all produced threshold pain in concentrations of 1 to 3 μM.

In animals electrophysiological evidence in support of sensory nerve stimulation has been obtained for potassium (Fenn, 1940; Brown & Gray, 1948), ACh (Brown & Gray, 1948; Douglas & Gray, 1953; Douglas & Ritchie, 1960; Fjällbrant & Iggo, 1961), 5-HT (Fjällbrant & Iggo, 1961; Beck & Handwerker, 1974) and bradykinin (Beck & Handwerker, 1974). Some information on the ability of ATP to stimulate sensory nerves has been obtained by Juan & Lembeck, (1974). Additional electrophysiological evidence that adenyl compounds other than ATP can stimulate sensory nerve fibres is desirable, and therefore objective studies of the sensory activities of the adenyl compounds were undertaken. This paper describes the electrophysiological effects of ATP, ADP and AMP as compared with those of potassium, ACh, 5-HT and bradykinin on cutaneous afferent nerve fibres of rabbits and cats.

Methods

Rabbit ear blister base preparation

The blistering agent cantharidin (0.3%) was applied to the shaved outer surface of one ear, close to its margin, in up to 5 areas. Each area measured approximately 16 mm^2 and was devoid of larger blood vessels. Three to five hours later, when cantharidin had raised a blister, the rabbit (of either sex and weighing 2.5 to 5 kg) was anaesthetized with urethane 28 to 34 mmol/kg, injected as a 2.2 M solution into an ear vein. Anaesthesia was maintained by injection, when required, of smaller doses of urethane via a cannula in the internal jugular vein on the side opposite to the blistered ear. The anaesthetized rabbit was placed so that the base of the blister was in a horizontal position and faced upwards. A mid-line incision was made along the axis of the ear on the dorsal side. The auricular-temporal nerve was exposed, de-

sheathed and then subdivided into bundles. Bundles in which mechanical stimulation of the intact blister initiated nerve action potentials (NAPs), were located and split into finer ones. From the latter, a bundle with minimal background activity was selected for subsequent recordings of NAPs. The blister base was then exposed by cutting away the epidermis above it. The exposed blister base was bathed continuously in blister-Ringer containing (mM): NaCl 157, KCl 5.4, $CaCl_2$ 2.2 and $NaHCO_3$ 1.8. The blister-Ringer was gassed with 95% O_2 and 5% CO_2; this gave a pH of 7.4. Experiments were performed on more distally located blisters first and when their responses to touch and test substances became reduced, more proximal blisters were used. In some experiments hyaluronidase 1500 iu/ml was applied to the blister base for 30 min before any test to increase the rate of diffusion. Substances dissolved in blister-Ringer, were applied to the blister base for 2 min every 10 minutes.

Cat skin preparation

The preparation was essentially set up as described by Fjällbrant & Iggo (1961). Cats of either sex, weighing 2 to 3 kg, were anaesthetized with chloralose (0.32 mmol/kg i.p.). Heparin (1500 iu) was injected via a cannula in the brachial vein, as were further small doses of chloralose required to maintain anaesthesia. The femoral and saphenous arteries were exposed by mid-thigh incision and a fine cannula, for close arterial injection of test substances, was inserted into one of the branches of the femoral artery, with its tip positioned just distal to the origin of the saphenous artery. All branches of the femoral artery other than the saphenous artery were ligated. Methylene blue was injected after each experiment to check that the blood supply to the skin was intact and restricted to it. During injections of test substances, the femoral artery was occluded centrally to the saphenous artery. Substances were injected in a volume of 0.2 ml followed by 0.1 ml 0.9% w/v NaCl solution (saline), after which normal blood flow was restored. The substances were administered at intervals of 15 min or more so as to allow nerve activity to return to its basal level subsequent to all injections. Nerves in which gentle stroking of the skin in the thigh or knee areas elicited NAPs were cut proximally, split into fine multifibre strands and NAPs recorded from them. In some experiments the blood pressure in the common carotid artery was monitored with a pressure transducer (Statham P23AA) coupled to a pen recorder (Devices M4).

Recording of nerve action potentials (NAPs)

Recordings were made from dissected multifibre strands of the nerve using saline wick electrodes. The nerve fibres were kept moist by intermittent application of isotonic glucose (0.34 M). The signals were amplified and simultaneously displayed on an oscilloscope and recorded with an ink jet recorder and on magnetic tape.

In all the experiments responses to application (rabbit) or injection (cat) of a similar volume of physiological solution were also tested. The response to mechanical stimulation by gently touching the blister base (rabbit) or skin (cat) with an insulated bristle was used as an indication of the stability of the recording conditions and the functional state of individual fibres.

Preparation of test samples

Unless otherwise stated, the pH of test samples was kept in the range of 6 to 8 by dissolving substances either in the physiological solution appropriate for the preparation under test, and where necessary, adding NaOH, or in 0.16 M Tris buffer (pH 7).

Solutions of test substances were made up on a weight/volume basis, but in this paper molar concentrations are given.

Drugs

The following drugs were used: acetylcholine chloride (Sigma), ATP, ADP, AMP, adenosine (Sigma), atro-

Table 1 Effects of substances that produce pain when applied to the human blister base on the rabbit ear blister base preparation

Substance tested	*Threshold and/or highest concentration applied*	*Onset of threshold concentration (s)*	*Duration range of threshold to highest concentration applied (s)*
KCl	13–134 mM (n = 46)	Immediate	5–60
ACh	55 mM (n = 4)	No response	—
Bradykinin	0.1 mM (n = 3)	No response	—
5-HT	25 mM (n = 7)	No response	—
AMP	23 mM (n = 4)	Immediate	40
ADP	4–18 mM (n = 8)	Immediate	10–60
ATP	16 mM (n = 4)	10	60

pine sulphate (BDH), bradykinin (Sandoz), 5-hydroxytryptamine (Koch Light), hyaluronidase-ovine (Fisons) and (+)-tubocurarine chloride (Koch Light).

Results

Rabbit ear blister base preparation

The results obtained when the various substances were applied to the rabbit ear blister base (76 experiments) are summarized in Table 1. It shows that potassium increased the frequency of NAPs at a threshold concentration of 13 mM. The effect, which was concentration-dependent, was immediate in onset and lasted for up to 60 seconds. However, acetylcholine, bradykinin and 5-HT in concentrations up to 55 mM, 0.1 mM and 25 mM, respectively did not initiate NAPs even when the blister base had been pretreated for 30 min with hyaluronidase.

Both ADP and ATP initiated NAPs in concentrations of 18 and 16 mM, respectively. The effect was sometimes delayed in onset by up to 10 s and lasted for up to 60 seconds. With ADP the concentration needed for a threshold effect is of the order of 4 mM. AMP gave a threshold effect at a concentration of 23 mM.

Cat skin preparation

The results obtained when the various substances were administered intra-arterially to anaesthetized cats (84 experiments) are summarized in Table 2. It shows that potassium increased the frequency of the NAPs is a dose range of 19–200 μmol. This effect was immediate in onset and lasted for up to 60 seconds. Acetylcholine produced a dose-dependent increase in the frequency of NAPs, with a threshold effect being given by 11 nmol. Responses to ACh were rapid in onset and lasted for a period of 0.3 to 6 minutes. To determine whether the NAPs elicited by ACh were induced indirectly through an action on blood vessels or striated muscle, ACh antagonists were used. Intravenous administration of atropine 1.6 μmol/kg blocked the vascular effect of 55 nmol

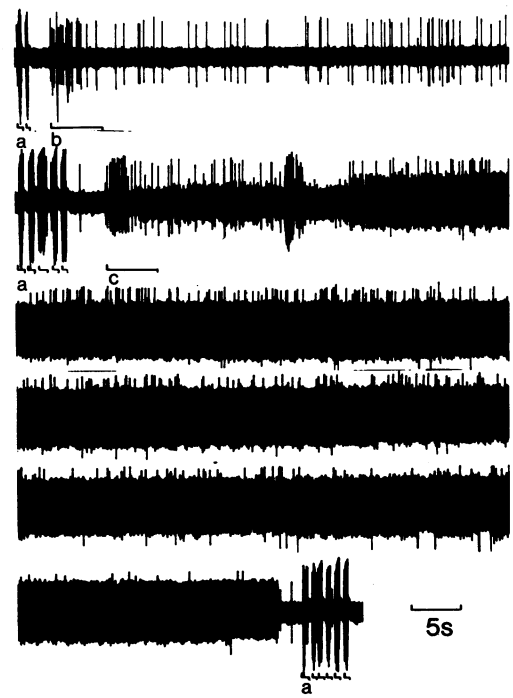

Figure 1 Nerve action potentials recorded from a multifibre strand of the saphenous nerve in an anaesthetized cat. (a) Intermittent touching of the skin with an insulated bristle. (b) Injection of 0.2 ml saline, pH 7, into the saphenous artery. (c) Injection of 18 μmol of ADP, dissolved in 0.2 ml saline, pH 7, into the saphenous artery. The effect is illustrated by four consecutive tracings. Touch and injections are denoted by horizontal bars.

Table 2 Effects of substances that produce pain when applied to the human blister base on the cat skin preparation

Substance tested	*Threshold and highest doses injected intra-arterially*	*Onset of threshold dose (s)*	*Duration range of threshold to highest dose injected (s)*
KCl	19–200 μmol (*n* = 19)	Immediate	15–60
ACh	11–55 nmol (*n* = 22)	Immediate	20–360
Bradykinin	2–20 nmol (*n* = 6)	Immediate	15–1020
5-HT	2.5–50 nmol (*n* = 17)	10	20–960
AMP	2.5–11.5 μmol (*n* = 5)	Immediate	180–300
ADP	2–18 μmol (*n* = 13)	Immediate-20	20–240
ATP	16 μmol (*n* = 2)	20	60

ACh, as indicated by blood pressure recording, without appreciably affecting the initiation of NAPs. (+)-Tubocurarine, 0.6 μmol/kg administered close arterially, abolished muscle fasciculations induced by ACh, but did not affect the initiation of NAPs. 5-HT elicited NAPs in a dose range of 2.5 to 50 nmol after a delay ranging from 5 to 60 s and its action lasted for a period of 0.3 to 16 minutes. Bradykinin increased the frequency of NAPs in doses of 2 and 20 nmol and the effect of the higher dose lasted approximately 17 minutes.

Table 2 also shows that ATP and ADP in a dose of 16 and 18 μmol, respectively, and 6 μmol AMP consistently initiated NAPs. The threshold dose for ADP is approximately 2 μmol. Responses to ADP and ATP on the whole had a latency of about 20 s and lasted for a period of 1 to 4 minutes. On the other hand, responses to AMP were immediate. Figure 1 shows a typical result obtained with ADP. ADP in doses which produced NAPs regularly also produced triphasic blood pressure changes. Pretreatment with atropine (1.6 μmol/kg, i.v.) and (+)-tubocurarine (0.6 μmol/kg, close arterially) had no effect on NAPs initiated by or vascular responses to a dose of 18 μmol of ADP.

When ADP or AMP were injected in combination with either ACh or 5-HT, all in doses which themselves produced only a threshold discharge of NAPs, the responses were greater than their individual effects, but there was no evidence of potentiation between adenyl compounds and ACh or 5-HT.

Discussion

These experiments show that all three adenine nucleotides, namely AMP, ADP and ATP, stimulate afferent nerve fibres. Adenosine has a limited water solubility and could only be tested in concentrations of up to 7.5 mM. These concentrations were found to be inactive.

On the rabbit blister base, a method which was modelled on the human blister base method (Armstrong, Dry, Keele & Markham, 1953) for pain evaluation, it was found that the adenine nucleotides and potassium were active, whilst the so-called algesic substances ACh, 5-HT and bradykinin were inactive even at much higher concentrations than those at which they were effective intra-arterially in the cat. This in part agrees with the observation of Burgess & Perl (1967) that in cats the topical application to abraded skin of heat or brakykinin failed to stimulate nociceptive nerves, but they also found strong acids to be ineffective; the latter is contrary to our findings (unpublished observations) where weak acids (pH 4) or strong acids (pH 1) did elicit nerve activity although the pH level is much lower than that which produces pain in man (Armstrong *et al.*, 1953).

Intra-arterially injected, the algesic substances exert their action on paravascularly located free sensory nerve endings serving as pain receptor sites (Lim, 1966; Juan & Lembeck, 1974) and the cat preparation utilizes this form of administration. In these experiments the algesic substances potassium, ACh, 5-HT and bradykinin were shown to be effective and in approximately the same concentration ranges as found by other workers (Brown & Gray, 1948; Douglas & Gray, 1953; Douglas & Ritchie, 1960; Fjällbrant & Iggo, 1961; Beck & Handwerker, 1974). In order of potency the adenine nucleotides ranked between 5-HT, bradykinin and ACh on the one hand and potassium, the least active, on the other. A similar stimulation of afferent nerves has been reported for intra-arterially administered ATP in rabbits (Juan & Lembeck, 1974).

The adenyl compounds share with ACh, 5-HT and bradykinin the ability to produce vasodilatation and it has been suggested that they may, together with these substances and potassium be mediators in exercise-induced vasodilatation. Release of adenyl compounds has been demonstrated on stimulation of a variety of muscle and nerve tissues both *in vivo* and *in vitro* (Holton, 1959; Abood, Koketsu & Miyamoto, 1962; Kuperman, Volpert & Okamoto, 1964; Forrester & Lind, 1969; Burnstock, 1972; McIlwain, 1972; Parkinson, 1973; Silinsky, 1975). Furthermore, the concentration of adenyl compounds increases when muscle stimulation or exercise are performed under hypoxic or ischaemic conditions (Rubio, Berne & Katori, 1969; Forrester, 1972; Forrester & Williams, 1977), circumstances in which noxious sensations and pain are experienced. According to Sicuteri (1968), the substances which regulate the microcirculation are identical with those which stimulate nociceptors. The demonstration of afferent nerve activity of the adenine nucleotides presented in this paper gives further evidence of their sensory stimulant effects.

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