Action of adenosine receptor antagonists on the cardiovascular response to defence area stimulation in the rat

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1 The action of adenosine in the mediation of the cardiovascular changes associated with the defence reaction has been investigated in the rat using two A₁ receptor antagonists.

2 Cumulative doses of 1,3 dipropyl-cyclopentylxanthine (DPCPX) (0.3–3 mg kg⁻¹) and ethanol (0.03–0.25 ml) and bolus doses of DPCPX (3 mg kg⁻¹) and 8-sulphophenyltheophylline (8-SPT) (20 mg kg⁻¹) were given into α -chloralose, paralysed and artificially ventilated rats. Recordings were made of arterial blood pressure and heart rate.

3 Ethanol, the vehicle for DPCPX, failed to modify the magnitude of the defence response; however, cumulative doses of DPCPX produced a dose-dependent decrease in the HDA (hypothalamic defence area)-evoked increase in arterial blood pressure, accompanied by a similar fall in the magnitude of the evoked heart rate response.

4 The evoked rise in arterial blood pressure was reduced significantly by intravenous injection of DPCPX (3 mg kg⁻¹) but not 8-SPT (20 mg kg⁻¹), a purely peripherally acting adenosine antagonist.

5 These results suggest that adenosine acting at A₁ receptors located in the central nervous system, is involved in the HDA-evoked pressor response. Whilst the site of action of the A₁ receptors is not known, possible locations are discussed.

Keywords: Adenosine A₁ receptors; hypothalamic defence area; 1,3 dipropyl-cyclopentylxanthine (DPCPX); 8-sulphophenyltheophylline (8-SPT); blood pressure; heart rate

Introduction

Adenosine, in addition to its function in intermediary metabolism, is, one of the major neuromodulators in the mammalian brain (see Stone, 1991, for review). It is locally and rapidly metabolized (Mudumbi *et al.*, 1993) and has numerous effects within the CNS and peripheral nervous system (see Contreras, 1990, for review). There is evidence suggesting a possible role of adenosine in the control of the cardiovascular system, often through its ability to modify the neural pathways related to the chemoreceptor and baroreceptor reflex responses (Mullane & Williams, 1990). A particular effect is the attenuation of the baroreceptor reflex (Hintze *et al.*, 1985). Further, there is evidence of an involvement of adenosine release in both the respiratory (hypoxic apnoea) and cardiovascular effects (secondary bradycardia and peripheral vasodilatation) observed during systemic hypoxia (Neylon & Marshall, 1991; Richter *et al.*, 1992).

Stimulation within the hypothalamic defence area in the cat produces circulatory changes which are partially mediated by the facilitation of the chemoreceptor reflex (Silva-Carvalho *et al.*, 1993) and an inhibition of the baroreceptor reflex, involving a GABAergic mechanism in the nucleus tractus solitarius (NTS) (Jordan *et al.*, 1988). These changes in baroreceptor and chemoreceptor reflex function resemble the effects of adenosine on these reflexes (see Silva-Carvalho *et al.*, 1993). Accordingly, it seemed reasonable to suggest that adenosine release might play a role in the cardiovascular and respiratory components of the defence response. Indeed, preliminary observations have indicated the involvement of adenosine in the pressor response to electrical stimulation within the hypothalamic defence area in the cat (Dawid-Milner *et al.*, 1993) and rat (St Lambert *et al.*, 1993).

Two major subclasses of adenosine receptors have been distinguished, A₁ and A₂ (Schwabe *et al.*, 1991), which are coupled to G-proteins and regulate several effector systems including adenylate cyclase, inositol phosphate and glucose transport (Merkel *et al.*, 1993). The object of this present study was to examine the potential role of adenosine A₁ receptors in the hypothalamic defence response. To examine the effect of adenosine on the defence response, two adenosine A₁ receptor antagonists were used. These are 1,3 dipropyl-cyclopentylxanthine (DPCPX), which acts both at a central and peripheral level (Williams, 1991), and 8-sulphophenyltheophylline (8-SPT), a purely peripherally acting non-specific adenosine antagonist (Daly, 1982).

Methods

Experiments were performed on 24 male Sprague Dawley rats (300–350 g). Anaesthesia was induced with sodium pentobarbitone (40 mg kg⁻¹, i.p.) and supplemented as necessary with α -chloralose (5 mg kg⁻¹, i.v.).

Surgical procedures

Catheters were inserted into a femoral artery for the measurement of arterial blood pressure and a femoral vein for the administration of drugs. The trachea was cannulated below the larynx and the animals breathed spontaneously until positioned in a stereotaxic frame, after which they were paralysed with gallamine triethiodide (4 mg kg⁻¹, i.v. supplemented with 3 mg kg⁻¹ h⁻¹) and artificially ventilated with O₂-enriched air. End tidal CO₂, recorded with a fast response CO₂ analyser (Analytical Development Company Ltd), was maintained in the range of 4–5 vol% by adjusting respiratory frequency. The ECG was recorded via a pre-amplifier and filter (Neurolog), from which heart rate was derived. Rectal temperature was maintained at 37–38°C by a

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servo-controlled heating pad. The depth of anaesthesia was assessed by observing the absence of changes in arterial blood pressure, heart rate and phrenic nerve activity to a paw pinch and supplementary doses of α -chloralose were given as required. In three cases blood flow to the hindlimb was assessed with an electromagnetic flow probe (Carolina Medical Electronics) placed around the femoral artery after ligation of the paw. The hypothalamus was stimulated (1 ms pulses, 50–200 μ A at 100 Hz for 5 s) with a concentric electrode (SNE 100, Rhodes Medical Electrodes). The hypothalamic defence area was located on the basis of the characteristic cardio-respiratory response observed in the rat (Yardley & Hilton, 1986).

Experimental protocol

Dose-related effects of DPCPX on the evoked blood pressure and heart rate response to hypothalamic stimulation were assessed. The effects of the A_1 antagonist, DPCPX (0.3–3.0 mg kg⁻¹) on the hypothalamic defence area evoked cardiovascular response was assessed by constructing a cumulative dose-response curve to the drug. DPCPX was administered cumulatively at 0.3, 0.7 and 2 mg kg⁻¹ doses in each rat. The drug was administered over a period of 5 min at intervals sufficient to allow blood pressure to a return to pre-stimulus resting values. Since DPCPX was dissolved in absolute ethanol, cumulative vehicle dose-response curves to ethanol (0.03–0.25 ml) were also constructed. The volume of absolute ethanol injected was the same as the volumes required to dissolve the various doses of DPCPX.

In the second part of the study, the effects of DPCPX and 8-SPT on the responses evoked on hypothalamic stimulation were compared. A single bolus dose of 8-SPT (20 mg kg⁻¹), ($n = 6$), or DPCPX (3 mg kg⁻¹), ($n = 6$), was administered intravenously 10 min before stimulating the hypothalamic defence area. In three of the animals in which a bolus dose of DPCPX (3 mg kg⁻¹) was administered, femoral blood flow was also measured in addition to the blood pressure and heart rate.

Histology

Stimulation sites were marked at the end of the experiment by passing 500 μ A d.c. current through the electrode for 10–20 s. Frozen sections of 100 μ m were cut at the level of the hypothalamus, counter stained with neutral red and examined under a light microscope for localization of stimulation sites. It should be noted that analysis was undertaken only after histological data had been obtained.

Analysis of data

In all experiments baseline values for mean arterial blood pressure and heart rate were measured immediately prior to hypothalamic defence area stimulation. As stimulation of the hypothalamic sites, corresponding to the defence area, resulted in a biphasic pressor response, changes in mean arterial blood pressure were divided into two categories, the primary and secondary phase. The primary response was assessed by measuring the peak rise in blood pressure observed during the 5 s stimulation of the hypothalamus. The secondary response was measured 5–10 s after the cessation of hypothalamic stimulation. Stimulus-evoked changes in heart rate were measured as the peak response observed during the 5 s stimulation of the defence area.

The effects of cumulative doses of DPCPX (0.3, 0.7 and 2 mg kg⁻¹) on baseline blood pressure and heart rate were compared with the response to the appropriate vehicle control (absolute ethanol, 0.03, 0.1 and 0.25 ml) at matched time intervals by two way analysis of variance and least significance difference for comparison between the means (Sokal & Rohlf, 1969). In addition, the effect of a bolus dose of DPCPX (3 mg kg⁻¹), ethanol (0.25 ml) and 8-SPT (20 mg

kg⁻¹) on baseline blood pressure and heart rate were analysed statistically by Student's paired *t* test. The effects of cumulative doses of DPCPX (0.1, 1 and 3 mg kg⁻¹) on the response to hypothalamic stimulation were then compared with the effects of the appropriate vehicle control at matched time intervals by two way analysis of variance and least significance difference for comparison between the means (Sokal & Rohlf, 1969). The effects of a bolus dose of DPCPX (3 mg kg⁻¹), ethanol (0.25 ml) and 8-SPT (20 mg kg⁻¹) on the blood pressure and heart rate responses were analysed statistically by Student's paired *t* test. All data are given as the means $\pm$ s.e.mean. Differences were considered significant when $P < 0.05$.

Drugs and solutions

The following compounds were used: gallamine triethiodide (Flaxedil) and sodium pentobarbitone (Sagatal) (May and Baker Ltd, Dagenham); 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) dissolved in 100% ethanol and 8-(*p*-sulphophenyl)theophylline (8-SPT), dissolved (20 mg) in 0.2 ml of 0.4 N NaOH then diluted in distilled water (Research Biochemicals Incorporated Natick); α -chloralose and di-sodium tetraborate 10-hydrate (BDH Ltd. Poole).

Results

The i.v. administration of ethanol ($n = 6$) and DPCPX ($n = 6$), caused only small and insignificant changes in baseline mean arterial blood pressure. Following administration of ethanol alone (0.03, 0.1 and 0.25 ml) small and insignificant changes from control values of heart rate were noted. However, DPCPX (0.3–3 mg kg⁻¹) caused a significant fall in heart rate at both 1 mg kg⁻¹ (-9 ± 5 beats min⁻¹) and 3 mg kg⁻¹ (-10 ± 3 beats min⁻¹) doses ($P < 0.01$).

Hypothalamic stimulation elicited a biphasic increase in blood pressure consisting of an initial increase in mean arterial blood pressure (Figure 2a and b), which was followed by a secondary elevation in pressure (Figure 2c and d) which continued for approximately 10–30 s after the cessation of stimulation (Figure 1). When applied cumulatively, DPCPX (0.3–3 mg kg⁻¹), induced a dose dependent reduction in the hypothalamic defence area-evoked increase in mean arterial blood pressure. A significant reduction occurred in both the primary (-12 ± 2 mmHg) and secondary phase (-22 ± 9 mmHg) of the response (Figures 1 and 2) at the highest dose ($P < 0.01$ and $P < 0.05$ respectively). In contrast, DPCPX had no significant effect on the magnitude of the tachycardia observed during hypothalamic stimulation at any dose. When applied alone, ethanol caused small yet statistically insignificant changes in the primary and secondary component of the pressor response (Figure 2) and in the heart rate response to hypothalamic defence area stimulation.

The i.v. bolus administration of DPCPX (3 mg kg⁻¹), ethanol (0.25 ml) and 8-SPT (20 mg kg⁻¹) failed to alter the resting values of mean arterial blood pressure (Figure 3). The bolus doses of ethanol and 8-SPT also failed to cause changes in baseline heart rate, whilst DPCPX (3 mg kg⁻¹) produced a significant increase (from 408.5 ± 30.1 to 428 ± 44.6 b.p.m., $P < 0.05$). A bolus injection of 8-SPT ($n = 6$) failed to alter the magnitude of the blood pressure and heart rate changes elicited on hypothalamic stimulation, whilst a subsequent bolus injection of DPCPX (3 mg kg⁻¹) ($n = 6$), caused a decrease in the primary phase of the evoked pressor response (from 58.1 ± 10.3 to 35.1 ± 10.8 mmHg, $P < 0.02$) but left the heart rate response unaffected (Figures 3 and 4). In the three experiments in which blood flow was measured, stimulation in this area of the hypothalamus elicited a marked increase (1.3 to 2.9 ml min⁻¹) in femoral blood flow. The administration of a bolus dose of DPCPX (3 mg kg⁻¹)

Figure 1 Typical recordings of arterial blood pressure and heart rate in an anaesthetized rat. The figure shows the effect of cumulative i.v. doses of ethanol (0.03–0.25 ml) and 1,3 dipropyl-cyclopentylxanthine (DPCPX, 0.3–3 mg kg⁻¹) on the pressor and heart rate responses evoked by 5 s hypothalamic defence area (HDA) stimulation.

Figure 2 Anaesthetized rats: the effect of cumulative i.v. doses of 1,3-dipropyl-cyclopentylxanthine (DPCPX) (0.3–3.0 mg kg⁻¹) and absolute ethanol (0.03–0.25 ml) on the absolute changes (Δ) (mmHg) in mean arterial blood pressure (BP) with respect to both the primary (1°), (a and b), and secondary (2°), (c and d), phases of the defence response. Each column shows the mean value (n = 6) with s.e. mean. These changes are compared with those changes caused by the vehicle, ethanol, by two way analysis of variance and the least significant difference test to compare the means. **P* < 0.05; ***P* < 0.01.

Figure 3 Anaesthetized rat: the effect of i.v. bolus doses of 8-sulphophenyltheophylline (8-SPT, 20 mg kg⁻¹) and 1,3 dipropyl-cyclopentylxanthine (DPCPX, 3 mg kg⁻¹) on baseline values of mean arterial blood pressure and heart rate and changes in mean arterial blood pressure and heart rate associated with the primary phase, evoked by hypothalamic defence area (HDA) stimulation. Each column shows the mean value ($n = 6$) with s.e.mean. * $P < 0.05$, ** $P < 0.02$.

Figure 4 Traces showing recordings of arterial blood pressure and heart rate in anaesthetized rat. The figure shows the effect of i.v. bolus doses of 8-sulphophenyltheophylline (8-SPT, 20 mg kg⁻¹) and 1,3 dipropyl-cyclopentylxanthine (DPCPX, 3 mg kg⁻¹) on the pressor and heart rate responses evoked by stimulation of the hypothalamic defence area (HDA) for 5 s.

reduced this increase somewhat (1.0–2.5 ml min⁻¹). However, we have insufficient observations to provide a quantitative assessment although a clear reduction in flow is seen in Figure 5 (compare a and b).

Histological analysis of all the above cases showed that the stimulation sites were in the region of the paraventricular nucleus of the hypothalamus (PVH) and the anterior hypothalamic area (AHA), 6.4 to 6.8 mm rostral to interaural level (Pellegrino *et al.*, 1979) (Figure 5c), overlapping the hypothalamic defence area as defined by Yardley & Hilton

(1986). In three cases (Figure 5c), whilst stimulation elicited a similar pattern of cardiovascular response (a rise in both blood pressure and heart rate), DPCPX was ineffective in reducing the magnitude of the evoked responses (increase in mean arterial pressure; from 49 to 48 mmHg). In all three cases the stimulation sites were ventral to the hypothalamic defence area (Figure 5c). These results were not included in the statistical evaluation of the action of adenosine antagonists on the responses induced by the stimulation of the hypothalamic defence area.

Figure 5 Traces showing a control recording (a) and the effect of an i.v. bolus dose of 1,3 dipropyl-cyclopentylxanthine (DPCPX, 3 mg kg⁻¹) (b) on heart rate, femoral blood flow and arterial blood pressure following the stimulation of the hypothalamic defence area. The distribution of histologically determined sites from which cardiovascular responses were elicited are illustrated in (c). The sections were taken from a level 6.8 mm rostral from interaural line. Stimulation sites where the evoked responses were antagonized by: (○) bolus dose of DPCPX (3 mg kg⁻¹); (□) 8-sulphonyltheophylline (20 mg kg⁻¹); (●) cumulative doses of DPCPX (0.3–3 mg kg⁻¹) and (■) cumulative doses of ethanol (0.03–0.25 ml) and stimulation sites where the evoked responses were unaffected by DPCPX (□).

Discussion

The present study has indicated that adenosine A₁ receptors, located in the central nervous system, are activated during the defence reaction that is evoked by electrical stimulation at restricted sites within the hypothalamus (Yardley & Hilton, 1986). The evoked cardiovascular response, which includes an increase in blood pressure and heart rate, was found to be reduced by the intravenous application of an DPCPX which is a highly potent and selective A₁ antagonist (Lohse *et al.*, 1987). It is known to act both peripherally and within the central nervous system (Williams, 1991). Conversely, the application of 8-SPT, an A₁/A₂ receptor antagonist, with a solely peripheral site of action (Daly, 1982), was ineffective in modifying the cardiovascular component of the hypothalamic defence response. Although 8-SPT is a broad spectrum adenosine antagonist, its *K_i* values indicate its higher affinity for the A₁ than A₂ receptor (*K_i* 2630 nM and 15,300 nM, respectively; RBI Handbook 1993). Our present observations on the dose-related effect of DPCPX required careful control as it was dissolved in ethanol, which on its own could cause changes in cardiovascular parameters. Sun & Reis (1992), and many other workers have suggested that ethanol decreases baroreceptor sensitivity. In the present study ethanol, in equivalent doses to those used as a vehicle, had no effect on resting levels of either mean arterial blood pressure or heart rate.

Stimulation of the hypothalamic defence area elicits a biphasic pressor response. It has been proposed that the two phases are mediated by different mechanisms, the primary phase being due to an increase in sympathetic tone, whilst the secondary phase is due to the release of catecholamines from the adrenal medulla (Eferakeya & Bunag, 1974). Whilst

8-SPT and ethanol failed to alter the magnitude of either component of the blood pressure response, DPCPX caused a dose-dependent decrease in both phases. Furthermore, its effect on the secondary phase was more prominent than on the primary phase. DPCPX was found ineffective in modulating the effects of stimulation at hypothalamic sites located ventral to the hypothalamic defence area. However stimulation at these sites had elicited similar changes in blood pressure and heart rate to those evoked on stimulating in the hypothalamic defence area. This implies, but does not prove, that the effects of DPCPX observed are linked specifically to the pathways descending from the hypothalamic defence area.

As yet, little is known of the neuropharmacology of the synaptic interactions that underlie the expression of the hypothalamic-induced defence response. There are indications, that the evoked cardiovascular response involves a centrally mediated attenuation of the baroreceptor reflex (see Coote *et al.*, 1972). This involves the activation of a GABAergic mechanism within the NTS that is directed to those NTS neurones that receive an excitatory baroreceptor input (Jordan *et al.*, 1988; Mifflin *et al.*, 1988b). Recent studies have also shown that the chemoreceptor reflex is facilitated during the defence reaction and this again involves an interaction between inputs at the level of the NTS (Silva-Carvalho *et al.*, 1993).

There is a growing literature indicating that adenosine, acting both peripherally and within the central nervous system, is involved in the control of respiration (Monteiro & Ribeiro, 1987; 1989; Wessberg *et al.*, 1988; Barraco & Janusz, 1989) and cardiovascular activity (de Burgh Daly, 1986; Tseng *et al.*, 1988). The present data indicate that adenosine A₁ receptors located in the central nervous system are

involved in the hypothalamic defence area-evoked pressor response. As yet the sites of adenosine action within the central nervous system are not resolved. Preliminary studies in the cat have shown that the magnitude of the cardiovascular response to baroreceptor and chemoreceptor stimulation may be modulated by the central action of DPCPX (Silva-Carvalho *et al.*, 1993). This A₁ antagonist appears to cause a facilitation of the baroreceptor reflex and a reduction in the chemoreceptor-evoked pressor response. Therefore it is attractive to speculate that this action of DPCPX involves effects at A₁ receptors located in the NTS since this nucleus plays a major role in the processing of both respiratory and cardiovascular reflexes (Jordan & Spyer, 1986) and is also involved in the expression of centrally evoked cardiovascular and respiratory responses (Mifflin *et al.*, 1988a). Another possible site of action is the rostroventrolateral medulla since

its sympatho-excitatory neurones are excited by stimulation within the hypothalamic defence area (McAllen, 1986a,b). This suggestion is strengthened by the fact that DPCPX produces a more profound effect on the pressor response observed during the defence response than on the accompanying tachycardia. The absence of an obvious effect of DPCPX and ethanol on respiratory evoked responses is also significant as it may well indicate that the site of A₁ receptors is at a point in the reflex pathways after which they have split into cardiovascular and respiratory components such as the rostroventrolateral medulla. Further experiments will be needed to resolve these issues.

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