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POTENTIATION OF THE EFFECTS OF ADENOSINE ON ISOLATED CARDIAC AND SMOOTH MUSCLE BY DIAZEPAM

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1 Adenosine (10^{-7} to 3×10^{-4} M) or 2-chloroadenosine (10^{-8} to 10^{-5} M) produced concentration-dependent inhibition of the responses of the rat isolated vas deferens to electrical field stimulation. In electrically driven (2 Hz) guinea-pig isolated left atria, adenosine (10^{-6} to 10^{-3} M) or 2-chloroadenosine (10^{-8} to 10^{-7} M) produced concentration-dependent decreases in isometric tension.

2 Diazepam (10^{-6} and 10^{-5} M) had no direct effect *per se*, but significantly potentiated the inhibitory action of adenosine on both tissues without altering the inhibitory effect of 2-chloroadenosine.

3 The adenosine uptake inhibitors, hydroxynitrobenzylthioguanosine (HNBTG, 10^{-5} M) and dipyridamole (10^{-5} M) also potentiated the inhibitory actions of adenosine in rat vas deferens, but not those of 2-chloroadenosine.

4 Following adenosine uptake inhibition in rat vas deferens by HNBTG (10^{-5} M), diazepam (10^{-5} M) failed to produce any significant further potentiation of the inhibitory action of adenosine.

5 It is concluded that the potentiation of adenosine by diazepam is possibly due to an inhibition of adenosine uptake.

Introduction

Diazepam (Valium) and other benzodiazepines have a wide therapeutic application as anxiolytics, anticonvulsants and muscle relaxants. Diazepam is commonly used in anaesthetic practice primarily for premedication or as a sedative during regional anaesthetic techniques. It has also been advocated as an intravenous induction agent.

The central actions of benzodiazepines are considered to involve an interaction with a specific, high affinity binding site which may result in a potentiation of the pre- and postsynaptic activities of γ-aminobutyric acid (GABA)-containing neurones (see review by Costa & Guidotti, 1979). Recently, an interaction between diazepam and adenosine has been reported. Firstly, it has been demonstrated that diazepam inhibits adenosine accumulation in brain slices (Mah & Daly, 1976). Secondly, the search for an endogenous ligand for the benzodiazepine binding sites revealed that the adenosine metabolites, inosine and hypoxanthine, could compete with diazepam for the binding sites (Skolnick, Marangos, Goodwin, Edwards & Paul, 1978). In addition, Phillis (1979) has shown that diazepam potentiates the adenosine-induced depression of electrical activity in cortical neurones *in vivo*. It was thus of interest to investigate whether diazepam also modified the peripheral actions of adenosine. The experiments described here examine the actions of diazepam *in vitro* on (a) the negative inotropic action of adenosine in guinea-pig atria and (b) the adenosine-mediated inhibition of neurotransmission in rat vas deferens (Clanachan, Johns & Paton, 1977). A preliminary account of some of these findings has been presented to the British Pharmacological Society (Clanachan & Marshall, 1978).

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Methods

Tissue preparations and recording of contractions

Male Hartley guinea-pigs (400 to 500 g) and Sprague-Dawley rats (150 to 200 g) were killed by a blow on the head followed by exsanguination.

Guinea-pig hearts were quickly removed and placed in oxygenated Krebs solution. Left atria were dissected free from ventricular tissue, suspended in organ baths (5 ml) containing Krebs solution at 34°C under a testing tension of 500 mg and allowed to stabilize for 30 min. They were electrically driven at 2 Hz (Grass stimulator, Model SD9), at twice threshold voltage (0.5 to 2.5 V) and pulse widths of 5 ms.

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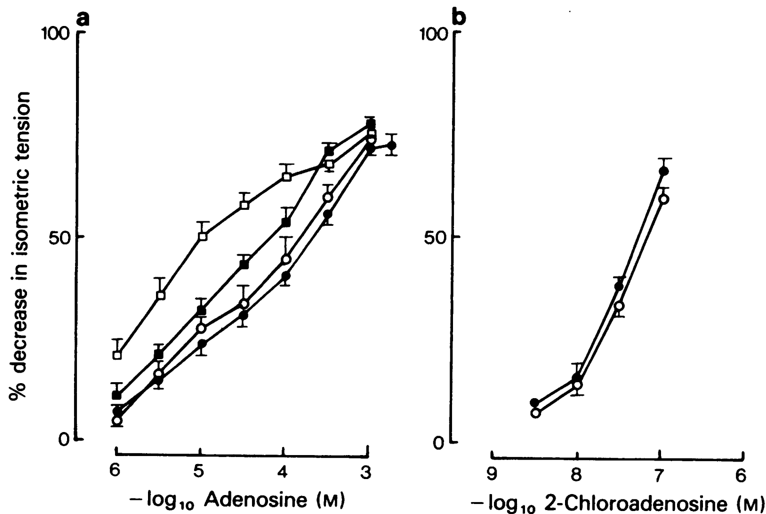

Figure 1 Effect of diazepam on the negative inotropic action of adenosine (a) and 2-chloroadenosine (b) on electrically driven (2 Hz) guinea-pig left atria. (a) Adenosine control (●), time control (○), 10^{-6} M diazepam (■) and 10^{-5} M diazepam (□); (b) 2-chloroadenosine control (●) and in presence of 10^{-5} M diazepam (○). Results are expressed as a mean % inhibition of the control isometric twitch response; vertical lines show s.e. mean, $n \geq 6$.

Rat vasa deferentia were removed and carefully dissected free from adhering tissue. Only the middle portion of each vas was used in this study. Vasa were prepared as described previously (Clanachan *et al.*, 1977) and contractions were elicited either by single pulses delivered every 30 s or by trains of pulses (5 Hz) for 30 s every 5 min (Grass stimulator, Model SM 6C).

Contractions of left atria and of vasa were recorded isometrically with force displacement transducers (Grass FT.03C) and displayed on a polygraph recorder (Grass Recorder, Model 7D).

Determination of concentration-effect curves

In atria cumulative concentration-effect curves to adenosine or 2-chloroadenosine were repeated at 20 min intervals until consistent responses were obtained (usually 3 times). Stepwise additions of drug were made at 1 min intervals. The curve was redetermined after 20 min incubation with either diazepam or its vehicle and compared to one obtained simultaneously in untreated tissue (time control). In vasa, non-cumulative concentration-effect curves were prepared for adenosine. Adenosine was in contact with the tissues for 1 min before electrical stimulation (5 Hz for 30 s). The curve was initially determined in normal Krebs solution. As the vasa are paired tissues, one then served as control (time control) whilst the other was exposed to the drugs. Diazepam and dipyridamole were prepared in Krebs solution and the tissues were exposed for 30 min before concentration-effect curves were redetermined. Hydroxynitrobenzylthioguanosine (HNBTG) was added directly to the organ baths to inhibit irreversibly adenosine uptake. Following a 30 min exposure period, HNBTG (10^{-5} M) was removed by washing the tissue with Krebs solution. Cumulative concentration-effect curves for 2-chloroadenosine were obtained in control and treated tissues.

For both atrial and vasa preparations, the mechanical response obtained after the addition of each succeeding concentration of adenosine or 2-chloroadenosine was expressed as a percentage of the initial response and the percentage inhibition calculated.

Statistical analysis of data

Results are expressed as mean ± standard error of the mean (s.e. mean). IC_{50} values (concentration producing 50% inhibition) were calculated by linear regression analysis. Significance levels for the difference between groups were estimated using Student's *t* test and the difference between groups was judged to be significant when $P < 0.05$.

Solution and drugs

The Krebs solution had the following composition (mM): NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22 and D-glucose, 11.2. The

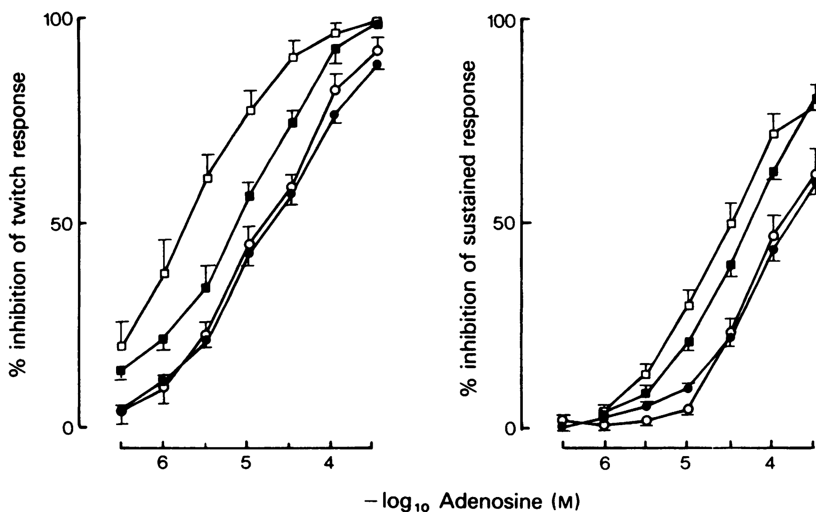

Figure 2 Effect of diazepam on the presynaptic inhibitory action of adenosine on the twitch (a) and sustained (b) response of the rat vas deferens. Control (●); time control (○); and in presence of 10^{-6} M diazepam (■) and 10^{-5} M diazepam (□). Results are expressed as mean % inhibition of response to electrical stimulation (5 Hz for 30 s); vertical lines show s.e. mean, $n = 6$.

solution was equilibrated with 95% O_2 and 5% CO_2 and maintained at 37°C (vas) or 34°C (atria).

Drugs used were: adenosine, 2-chloroadenosine (Sigma Chemical Co.); diazepam (Valium, Hoffmann-LaRoche Ltd.); dipyridamole (Boehringer-Ingelheim); HNBTG, 2-amino-6-[2-hydroxy-5-nitro) benzylthio]-9-β-D-ribofuranosyl purine (kindly donated by Dr A.R.P. Paterson, University of Alberta Cancer Research Unit); methacholine (Sigma).

Results

Effect of diazepam on adenosine and 2-chloroadenosine action in guinea-pig atria

Adenosine (10^{-6} to 10^{-3} M) and 2-chloroadenosine (10^{-8} to 10^{-7} M) caused concentration-dependent reductions of the isometric tension of electrically driven (2 Hz) guinea-pig left atria. Adenosine ($IC_{50} = 191 \pm 37$ μM, $n = 24$) was significantly ($P < 0.001$) less potent than 2-chloroadenosine ($IC_{50} = 50 \pm 5$ nM, $n = 7$). In concentrations which had no direct effect on atrial contractions, diazepam (10^{-6} and 10^{-5} M) significantly potentiated (3.4 times, $P < 0.05$ and 16.5 times, $P < 0.001$, respectively) the negative inotropic action of adenosine whereas the effect of 2-chloroadenosine was unaffected (Figure 1), values for potentiation being derived from the reduced IC_{50} values. The potentiating effects of diazepam were reversible on washing the tissues for 15 to 30 min. Diazepam (10^{-5} M) did not affect the negative inotropic effects of methacholine, the IC_{50} being 0.14 ± 0.02 μM ($n = 6$) before and 0.11 ± 0.03 μM ($n = 6$) 20 min after diazepam.

Effect of diazepam on adenosine and 2-chloroadenosine action in rat vas deferens

Diazepam (10^{-6} to 10^{-5} M) had no effect on the resting tension of rat vasa. Electrical field stimulation with trains of pulses elicited responses which displayed a characteristic initial twitch response followed by a secondary, sustained contraction. Frequency-response curves obtained for both the twitch and sustained responses in the presence of diazepam (10^{-5} M) were not significantly different from controls.

As has been shown previously (Clanachan & Paton, 1977; Clanachan *et al.*, 1977), adenosine (10^{-7} to 3×10^{-4} M) inhibited both the twitch ($IC_{50} = 23 \pm 3$ μM, $n = 20$) and sustained ($IC_{50} = 191 \pm 22$ μM, $n = 20$) responses of the rat vas following electrical field stimulation at 5 Hz (Figure 2). Previous studies have demonstrated that this inhibition is due to a decrease in sympathetic transmitter release (Clanachan, 1979).

Successive adenosine concentration-effect curves indicated that the twitch response of the rat vas deferens displays a small degree of sensitization to the presynaptic inhibitory action of adenosine. IC_{50} values for the second concentration-effect curve (time control) were 15 ± 2 μM ($n = 10$) and 185 ± 32 μM

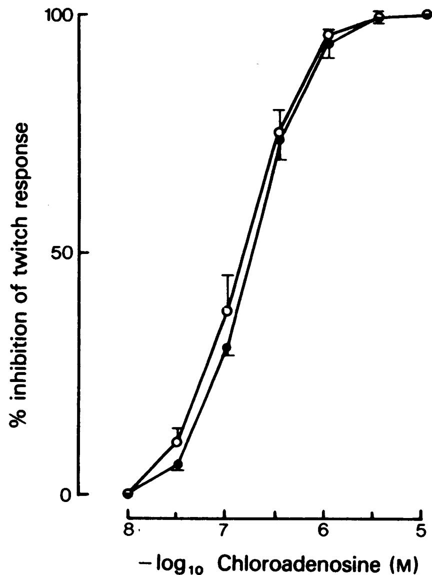

Figure 3. Effect of diazepam on the presynaptic inhibitory action of 2-chloroadenosine on the twitch response of the rat vas deferens. Control (●) and in the presence of 10^{-5} M diazepam (○). Results are expressed as mean % inhibition of the response to electrical stimulation (single pulse every 30 s); vertical lines show s.e. mean, $n = 5$.

($n = 10$) for the twitch and sustained responses respectively. In order to account for this sensitization, the effect of the various treatments on adenosine sensitivity were compared with their respective time control IC_{50} value.

Diazepam (10^{-6} and 10^{-5} M) significantly potentiated the presynaptic inhibitory action of adenosine on both the twitch ($IC_{50} = 6 \pm 1$ μM $P < 0.01$, $n = 6$ and 2 ± 1 μM, $P < 0.001$, $n = 6$, respectively) and sustained response ($IC_{50} = 53 \pm 6$ μM, $n = 6$ and 45 ± 17 μM, $n = 6$, respectively). The potentiation of adenosine action by diazepam was dose-dependent (Figure 2) and was easily reversed on washing the preparation with Krebs solution.

2-Chloroadenosine has also been shown to inhibit responses of the rat vas deferens to electrical field stimulation by a presynaptic mechanism. Its greater apparent potency compared with adenosine is presumably due to its inability to act as a substrate for the adenosine uptake system (Muller & Paton, 1979). In the present experiments, 2-chloroadenosine (10^{-8} to 10^{-5} M) produced a dose-dependent inhibition ($IC_{50} = 0.18 \pm 0.01$ μM, $n = 6$) of contractile responses to single pulses of electrical field stimulation (Figure 3). However, in contrast to the inhibition by adenosine, inhibition by 2-chloroadenosine was not potentiated by 10^{-5} M diazepam ($IC_{50} = 0.18 \pm 0.04$ μM, $n = 5$).

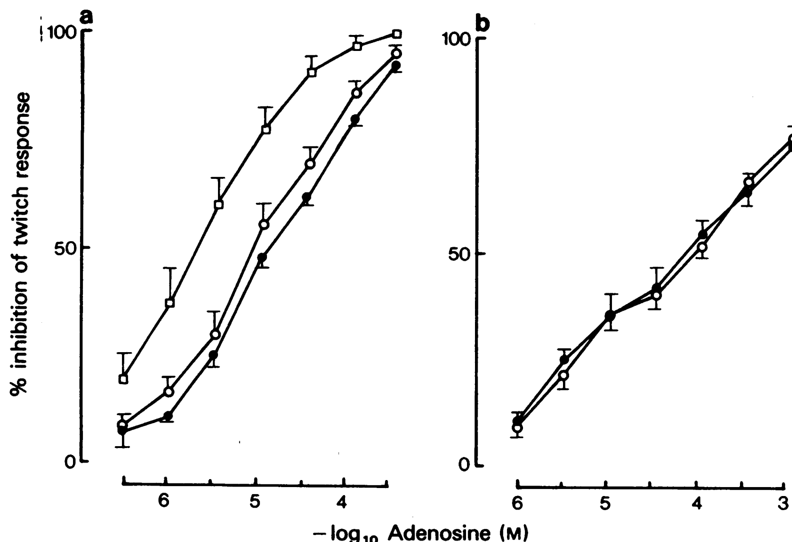

Figure 4 Effect of diazepam solvent, in a concentration equivalent to that present in a 10^{-5} M diazepam solution, on the presynaptic inhibition of responses to electrical stimulation (5 Hz) by adenosine (a) and on the negative inotropic action of adenosine in electrically driven (2 Hz) guinea-pig left atria (b). Adenosine control (●) and in the presence of diazepam solvent (○). The effect of 10^{-5} M diazepam on adenosine effects in rat vas deferens is shown for comparison (□). Results are expressed as the mean % of inhibition of the response; vertical lines show s.e. mean, $n = 6$.

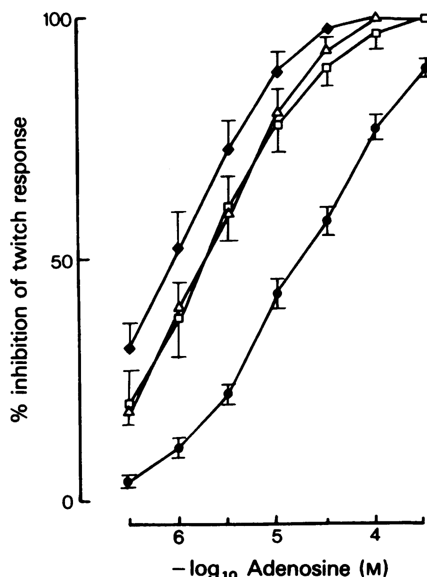

Figure 5 Effect of an adenosine uptake inhibitor, hydroxynitrobenzylthioguanosine (HNBTG) on the potentiation of the presynaptic inhibitory action of adenosine in rat vas deferens. Adenosine control (●) and in the presence of 10^{-5} M diazepam (□); following adenosine uptake inhibition by 10^{-5} M HNBTG (△); and in the presence of 10^{-5} M diazepam following adenosine uptake inhibition by 10^{-5} M HNBTG (◆). Results are expressed as mean % inhibition of the twitch response of the rat vas deferens to electrical stimulation (5 Hz for 30 s). Results concerning changes of the sustained response were similar.

Effect of diazepam solvent on adenosine and 2-chloroadenosine action to guinea-pig atria and rat vas deferens.

Many investigators have reported that the commonly used solvent for diazepam (propylene–glycol and ethyl alcohol) exerts significant pharmacological activity. However, in the present experiments (Fig. 4), a concentration of solvent equivalent to that present following preparation of the 10^{-5} M diazepam in Krebs solution produced a small but insignificant potentiation of inhibitory action of adenosine. IC_{50} values for adenosine were not significantly different from corresponding time control values (Figure 4).

Effect of adenosine uptake inhibitors on the presynaptic inhibitory actions of adenosine and 2-chloroadenosine in rat vas deferens

HNBTG is a potent, irreversible inhibitor of adenosine uptake. Pretreatment of vasa with HNTBG (10^{-5} M for 30 min) has been shown to potentiate significantly the effects of adenosine (Clanachan *et al.*, 1977) but not those of 2-chloroadenosine (Muller & Paton, 1979). These results were confirmed in the present study. Similarly, dipyridamole (10^{-5} M), another although less specific inhibitor of adenosine uptake (Baer, Frew & Burnstock, 1977), also potentiated the effect of adenosine but not that of 2-chloroadenosine. A similar potentiation of the inhibitory effect of adenosine in vas deferens by HNBTG, dipyridamole and diazepam is observed at both stimulation frequencies (5 Hz and 0.03 Hz) used in the present study (Muller & Paton, 1979; Clanachan & Muller, 1980).

More important, however, was the finding that following adenosine uptake inhibition by HNTBG, diazepam (10^{-5} M) failed to produce any significant further potentiation of the inhibitory effects of adenosine (Figure 5). The IC_{50} of adenosine on the twitch response following treatment with HNBTG was 2.2 ± 0.5 μM, whereas the IC_{50} in the presence of 10^{-5} M diazepam following treatment with HNBTG was 1.1 ± 0.3 μM ($0.97 > P > 0.95$).

Discussion

The main central effect of diazepam and other benzodiazepines is considered to involve GABA or perhaps another endogenous ligand with affinity for benzodiazepine binding sites (Gallager, Thomas & Tallman, 1978). It has also been shown that purine derivatives are powerful inhibitors of central neurones (Phillis & Edstrom, 1976). Since endogenous adenosine can be released in the CNS (McIlwain, 1972) diazepam may also act by potentiating an inhibitory neuromodulating action of adenosine (Phillis, 1979). A role for purines in diazepam action is also implied by the observation that the adenosine metabolites, inosine and hypoxanthine, can compete for the benzodiazepine binding sites (Skolnick *et al.*, 1978). The present study extends these observations and has demonstrated that a diazepam-adenosine interaction exists in peripheral neuroeffector systems. Diazepam has now been shown to potentiate the action of adenosine in cardiac muscle (guinea-pig atria) and in a smooth muscle preparation (rat vas deferens).

Diazepam (10^{-6} or 10^{-5} M) had no direct effect on the responses of atria or vasa to electrical stimulation which is consistent with its minimal peripheral depressant effects. Higher concentrations (10^{-4} M), however, do have nonspecific depressant effects on noradrenergic transmission (Bradshaw, 1976). In the present study, care was taken to ensure that the concentrations used were in the range of those expected clinically. Following intravenous administration of diazepam (20 mg) a serum concentration of approximately 6×10^{-6} M (1607 ng/ml) can be obtained (Hil-

lestad, Hansen Melsom & Drivenes, 1974). Four hours after intravenous, intramuscular or oral administration of diazepam (20 mg), serum concentrations are about 10^{-6} M (225 to 325 ng/ml). Much higher concentrations of diazepam can be expected following doses of diazepam (0.8 mg/kg) that have been associated with the induction of anaesthesia (Rolly, 1976).

Adenosine and other purine compounds (Paton, Baer, Clanachan & Lauzon, 1978) have been shown to inhibit autonomic neurotransmission by a presynaptic mechanism. The nerve-evoked release of [^{3}H]-NA from rat vas deferens is reduced by adenine nucleotides and adenosine (Clanachan *et al.*, 1977). Similar findings have now been reported in many other tissues (Enero & Saidman, 1977; Verhaeghe, Vanhoutte & Shepard, 1977; Su, 1978; Wakade & Wakade, 1978; Hedqvist & Fredholm, 1979; Meuller, Mosimann & Weiner, 1979). The release of acetylcholine from parasympathetic nerves is also inhibited by adenosine (see e.g. Sawynok & Jhamadas, 1976).

2-Chloroadenosine was significantly more potent than adenosine on both atrial and vasa preparations. However, as adenosine and 2-chloroadenosine were tested on vasa responses elicited by different frequencies of electrical stimulation (5 Hz and 0.03 Hz, respectively) an absolute potency ratio cannot be obtained. However, in previous studies in which 2-chloroadenosine and adenosine have been compared at the same stimulation frequency, 2-chloroadenosine was approximately 100 times more potent than adenosine (Muller & Paton, 1979; Clanachan & Muller, 1980).

In atria, 2-chloroadenosine also displayed a greater activity than adenosine and was approximately 3800 times more potent. The different relative potencies of these agonists in vasa and atria is probably related to a difference in their routes of inactivation in the two tissues, e.g., in vasa the potency of adenosine is masked by rapid uptake (Clanachan *et al.*, 1977) and deamination (Muller & Paton, 1979) of the nucleoside.

The presynaptic effect of adenosine on rat vas deferens was potentiated by diazepam (10^{-6} and 10^{-5} M). This effect is not due to a non-specific depressant action on the neurotransmission process as responses to 2-chloroadenosine were unchanged. The potentiation by diazepam was dose-dependent and easily reversible. Although the commercial solvent of diazepam (propylene–glycol and ethyl alcohol) has been reported to exert significant pharmacological activity in some systems (Crankshaw & Raper, 1971), it appears that solvent effects are not involved to any great extent in this study as the solvent alone, in an equivalent concentration to that used with diazepam, only exerted a minor effect on adenosine action.

Similarly, in atria, the negative inotropic action of adenosine, but not of 2-chloradenosine or metacholine was potentiated by diazepam. Also, the solvent exerted no significant effect on the cardiac actions of adenosine.

A plausible mechanism for the potentiation by diazepam of the inhibitory effect of adenosine may be that the uptake, and hence inactivation, of adenosine was inhibited. This suggestion is supported by the observation that the presynaptic inhibitory action of 2-chloroadenosine, an adenosine analogue which appears not to be a substrate for the adenosine uptake system, is not potentiated by diazepam. Furthermore, in the presence of dipyridamole or HNBTG, adenosine uptake inhibitors which increase adenosine action in many systems including cardiac (Stafford, 1966; Olsson, Snow, Gentry & Frick, 1972) and smooth muscle (Kalsner, 1975; Coleman, 1976; Clanachan *et al.*, 1977), diazepam produced no further significant potentiation of adenosine effects.

As diazepam had no direct effect on the neurotransmission process in rat vas deferens, it appears that adenosine may not be involved in the normal control of the peripheral neurotransmission process *in vitro*. However, under conditions where the release of endogenous adenosine is stimulated, drugs such as diazepam may increase adenosine effects.

Adenosine may be the physiological mediator of coronary vasodilatation in response to changes in myocardial oxygen requirement (Berne, 1963; Berne, Rubio, Dobson & Curnish, 1971). It is noteworthy that diazepam exerts considerable coronary vasodilator activity (Abel, Reis, & Staroscik, 1970a; 1970b; Daniell, 1975) which is greater in patients with ischaemic heart disease (Ikram, Rubin & Jewkes, 1973). Previous investigations intent on elucidating the mechanism underlying this coronary vasodilator activity of diazepam revealed that the mechanism was independent of systemic effects and required that diazepam be delivered to the coronary circulation (Abel *et al.*, 1970). It is tempting to speculate that diazepam increases coronary blood flow by an action similar to that of dipyridamole, namely adenosine uptake inhibition. We have shown that diazepam also potentiates the coronary vasodilator actions of adenosine in anaesthetized dogs (Clanachan & Marshall, 1980). As diazepam is widely used in patients with ischaemic heart disease, further investigation of this aspect of diazepam action is warranted since direct coronary vasodilatation such as that produced by adenosine, dipyridamole, dilazep, hexabendine and lidoflazine has been considered to compromise perfusion of ischaemic areas by 'coronary steal', i.e. diversion of blood away from poorly perfused, but maximally dilated areas (Marshall & Parratt, 1973).

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References

ABEL, R.M., REIS, R.L. & STAROSCIK, R.N. (1970a). Coronary vasodilation following diazepam (Valium). *Br. J. Pharmac.*, **38**, 620–631.

ABEL, R.M., REIS, R.L. & STAROSCIK, R.M. (1970b). The pharmacological basis of coronary and systemic vasodilator actions of diazepam (Valium). *Br. J. Pharmac.*, **39**, 261–274.

BAER, H.P., FREW, R. & BURNSTOCK, G. (1977). Effects of dipyridamole and 6-(2-hydroxy-5-nitro)-benzylthioguanosine on low-frequency-stimulated relaxation in the guinea-pig taenia coli. *Can. J. Physiol. Pharmac.*, **55**, 394–398.

BERNE, R.M. (1963). Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *Amer. J. Physiol.*, **204**, 317–322.

BERNE, R.M., RUBIO, R., DOBSON, J.G. & CURNISH, R.R. (1971). Adenosine and adenine nucleotides as possible mediators of cardiac and skeletal muscle blood flow regulation. *Circulation Res.*, **28**, Suppl. 1, 115–119.

BRADSHAW, E.G. (1976). The vasodilator effects of diazepam *in vitro*. *Br. J. Anaesth.*, **48**, 817–818.

CLANACHAN, A.S. (1979). Modification of release by adenosine and adenine nucleotides. In *The Release of Catecholamines from Adrenergic Nerves* ed. Paton, D.M. pp. 263–273. Oxford: Pergamon Press.

CLANACHAN, A.S., JOHNS, A. & PATON, D.M. (1977). Inhibitory actions of adenine nucleotides and adenosine on neurotransmission in rat vas deferens. *Neuroscience*, **2**, 597–602.

CLANACHAN, A.S. & MARSHALL, R.J. (1978). Therapeutic concentrations of diazepam potentiate the effects of adenosine on isolated cardiac and smooth muscle. *Br. J. Pharmac.*, **68**, 148P.

CLANACHAN, A.S. & MARSHALL, R.J. (1980). Diazepam potentiates the coronary vasodilator actions of adenosine in anaesthetized dogs. *Br. J. Pharmac.*, (in press).

CLANACHAN, A.S. & MULLER, M.H. (1980). Effect of adenosine uptake inhibition on the nature and potency of theophylline as a presynaptic adenosine receptor antagonist. *Can. J. Physiol. Pharmac.*, **58**, 805–809.

CLANACHAN, A.S. & PATON, D.M. (1977). Inhibitory actions of adenine nucleotides and adenosine on transmission in rat vas deferens. *Br. J. Pharmac.*, **59**, 534P.

COLEMAN, R.A. (1976). Effects of some purine derivatives on the guinea-pig trachea and their interaction with drugs that block adenosine uptake. *Br. J. Pharmac.*, **57**, 51–57.

COSTA, E. & GUIDOTTI, A. (1979). Molecular mechanisms in the receptor action of benzodiazepines. *A. Rev. Pharmac. Tox.*, **19**, 531–545.

CRANKSHAW, D.P. & RAPER, C. (1971). The effect of solvents on the potency of chlordiazepoxide, diazepam, medazepam and nitrazepam. *J. Pharm. Pharmac.*, **23**, 313.

DANIELL, H.B. (1975). Cardiovascular effects of diazepam and chlordiazepoxide. *Eur. J. Pharmac.*, **32**, 58–65.

ENERO, M.A. & SAIDMAN, B.Q. (1977). Possible feedback inhibition of noradrenaline release by purine compounds. *Naunyn Schmiedebergs Arch. Pharmac.*, **297**, 39–46.

GALLAGER, B.W., THOMAS, J.W. & TALLMAN, J.F. (1978). Effect of gabaergic drugs on benzodiazepine binding site sensitivity in rat cerebral cortex. *Biochem. Pharmac.*, **27**, 2745–2749.

HEDQVIST, P. & FREDHOLM, B.B. (1979). Inhibitory effect of adenosine on adrenergic neuroeffector transmission in the rabbit heart. *Acta. physiol. scand.*, **105**, 120–122.

HILLESTAD, L., HANSEN, T., MELSOM, H. & DRIVENES, A. (1974). Diazepam metabolism in normal man—I. Serum concentrations and clinical effects after intravenous, intramuscular and oral administration. *Clin. Pharmac. Ther.*, **16**, 479–484.

IKRAM, H., RUBIN, A.P. & JEWKES, R.F. (1973). Effect of diazepam on myocardial blood flow of patients with and without coronary artery disease. *Br. Heart J.*, **35**, 626–630.

KALSNER, S. (1975). Adenosine and dipyridamole actions and interactions on isolated coronary artery strips of cattle. *Br. J. Pharmac.*, **55**, 439–445.

MAH, H.D. & DALY, J.W. (1976). Adenosine-dependent formation of cyclic AMP in brain slices. *Pharmac. Res. Commun.*, **8**, 65–79.

MARSHALL, R.J. & PARRATT, J. (1973). The effects of dipyridamole on blood flow and oxygen handling in the acutely ischaemic and normal canine myocardium. *Br. J. Pharmac.*, **49**, 391–399.

MCILWAIN, H. (1972). Regulatory significance of the release and action of adenine derivatives in cerebral system. *Biochem. Soc. Symp.*, **36**, 69–85.

MEULLER, A.L., MOSIMANN, W.F. & WEINER, N. (1979). Effects of adenosine on neurally mediated noradrenaline release from the cat spleen. *Eur. J. Pharmac.*, **53**, 329–335.

MULLER, M.J. & PATON, D.M. (1979). Presynaptic inhibition by 2-substituted adenosine derivatives in the rat vas deferens: studies with adenosine uptake and deaminase inhibitors. *Naunyn Schmiedebergs Arch. Pharmac.*, **306**, 23–28.

OLSSON, R.A., SNOW, J.A., GENTRY, M.K. & FRICK, P. (1972). Adenosine uptake by canine heart. *Circulation Res.*, **31**, 767–778.

PATON, D.M., BAER, H.P., CLANACHAN, A.S. & LAUZON, P. (1978). Structure activity relations for inhibition of neurotransmission in rat vas deferens by adenosine. *Neuroscience*, **3**, 65–70.

PHILLIS, J.W. (1979). Diazepam potentiation of purinergic depression of central neurones. *Can J. Physiol. Pharmac.*, **57**, 432–435.

PHILLIS, J.W. & EDSTROM, J.P. (1976). Effects of adenosine analogs on rat cerebral cortical neurones. *Life Sci.* **19**, 1041–1054.

ROLLY, G. (1976). Diazepam–lorazepam and Flunitrazepam. In *Anaesthesia and Pharmacology*. ed. Spierdijk, J., Feldman, S.A. & Mattie, H., pp. 127–136. Baltimore: The William and Wilkins Co.

SAWYNOK, J. & JHAMANDAS, K.H. (1976). Inhibition of acetylcholine release from cholinergic nerves by adenosine, adenine nucleotides and morphine: antagonism by theophylline. *J. Pharmac. exp. Ther.*, **197**, 379–390.

SKOLNICK, P., MARANGOS, P.J., GOODWIN, F.K., EDWARDS, M. & PAUL, S. (1978). Identification of inosine and hypoxanthine as endogenous inhibitors of [^{3}H]diazepam binding in the central nervous system. *Life Sci.*, **23**, 1473–1480.

STAFFORD, A. (1966). Potentiation of adenosine and the adenine nucleotides by dipyridamole. *Br. J. Pharmac. Chemother.*, **28**, 218–227.

SU, C. (1978). Purinergic inhibition of adrenergic transmission in rabbit blood vessels. *J. Pharmac. exp. Ther.*, **204**, 351–401.

VERHAEGHE, R.H., VANHOUTTE, P.M. & SHEPARD, J.T. (1977). Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides. *Circulation Res.*, **40**, 208–215.

WAKADE, A.R. & WAKADE, T.O. (1978). Inhibition of noradrenaline release by adenosine. *J. Physiol.*, **282**, 35–49.

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