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Adenosine Affects Sympathetic Neurotransmission at Multiple Sites *in Vivo*[1]

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ABSTRACT

We examined the effects of adenosine and its analogs on sympathomimetic responses of pithed rats to electrical stimulation of preganglionic sympathetic nerves (ES) or to injections of nicotine, phenylephrine (PE) or isoproterenol (ISO). Four physiological indices of sympathetic neurotransmission were measured: blood pressure, heart rate and contractions of smooth muscle in vas deferens and eyelid. Elevation of arterial adenosine levels from 1.5 to 2 to 3 μM caused a 2- to 3-fold potentiation of nicotine-induced increases in blood pressure, heart rate and smooth muscle tension. Higher adenosine concentrations (3–4 μM) produced a smaller potentiation of the effects of nicotine. At 2 to 3 μM, adenosine had no effect on sympathomimetic responses to ES or PE. Higher concentrations (3–4 μM) attenuated pressor responses to ES and PE and the contractile responses of the vas deferens to ES; these levels also potentiated positive chronotropic responses to ISO. The adenosine analogs N-cyclopropylcarboxamido adenosine (N-CPCA), 2-chloroadenosine (2-CLA) and *R*- and *S*-phenylisopropyl adenosine (*R*-PIA and *S*-PIA) also reduced pressor responses to both ES and PE, with the potency order: N-CPCA > *R*-PIA > 2-CLA > *S*-PIA. These analogs exhibited this same potency series in attenuating contractile responses to ES in the vas deferens. However, all four analogs potentiated, at the lower doses tested, the contractile response of the vas deferens to PE; at higher concentrations, inhibition predominated. N-CPCA enhanced the chronotropic effects of ISO and ES. Circulating adenosine, at levels within or near the physiological range, or synthetic adenosine analogs, can influence sympathetic transmission *in vivo* by interacting with at least three sites: sympathetic ganglia, sympathetic nerve terminals and sympathetically innervated end organs.

The purine nucleoside adenosine can modulate neurotransmission by interacting with specific receptors on pre- or postsynaptic tissues (Daly *et al.*, 1981). Presynaptically, adenosine inhibits the release of several neurotransmitters (Fredholm and Hedqvist, 1980); postsynaptically it can either inhibit or potentiate the actions of neurotransmitters depending on the tissue and its concentration, particularly in tissues containing multiple subpopulations of adenosine receptors mediating different processes (Van Calker *et al.*, 1979). For example, in neural tissue low concentrations of adenosine inhibit isoproterenol-induced activation of adenylate cyclase *via* A1 receptors, whereas higher concentrations activate adenylate cyclase directly *via* A2 receptors, potentiating the stimulatory action of norepinephrine and other monoamines on cyclic AMP formation (Van Calker *et al.*, 1979; Londos *et al.*, 1980; Mah and Daly, 1976). Adenosine may exert opposing influences upon neurotransmission at presynaptic *vs.* postsynaptic sites within the same synaptic population. For example, in the isolated rabbit kidney, adenosine simultaneously reduces evoked release of norepinephrine from renal vascular sympathetic terminals and enhances vascular responsiveness to norepinephrine; its net influence is to enhance vasoconstrictor responses to renal nerve stimulation, even though the release of norepinephrine during nerve stimulation is decreased (Hedqvist *et al.*, 1978). Because adenosine is normally present in plasma (Pritchard *et al.*, 1975; von Borstel *et al.*, 1983) and can cross the blood-brain barrier (Cornford and Oldendorf, 1975), the nucleoside could conceivably act as a neuromodulator at a number of different sites, affecting neurotransmission and therein altering physiological functions, including blood pressure or heart rate.

We described recently a novel effect of adenosine on pressor responses to pharmacologic stimuli in the rat (von Borstel *et al.*, 1984). Very small increases in plasma adenosine concentrations, or low doses of its synthetic analogs, potentiated the effects of nicotine on ganglionic transmission dramatically, strongly enhancing such sympathomimetic effects of injected or inhaled (in cigarette smoke) nicotine as hypertension and

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ABBREVIATIONS: SNS, sympathetic nervous system; PE, phenylephrine; ISO, isoproterenol; PIA, phenylisopropyladenosine; CPCA, cyclopropylcarboxamidoadenosine; 2-CLA, 2-chloroadenosine; ES, preganglionic electrical stimulation.

tachycardia (von Borstel *et al.*, 1984; 1986). This finding contrasts with the well-established tendency of adenosine to attenuate systemic cardiovascular responses to sympathetic nerve stimulation (Lokhandwala, 1979; Hom and Lokhandwala, 1981; Kamikawa *et al.*, 1980). The present studies were designed to determine: 1) the net effect of adenosine on sympathomimetic responses *in vivo*; 2) the relative contributions of the actions of adenosine at various SNS loci to its overall effects on physiological functions; and 3) the concentrations of plasma adenosine needed to produce such effects. In these studies, we produced controlled and sustained elevations in circulating adenosine concentrations, using a slow i.v. infusion. Adenosine levels were thereby elevated incrementally, allowing determination of its relative potencies at various loci within the efferent SNS. We examined the abilities of adenosine, and of some of its derivatives, to influence postsynaptic elements of sympathetic transmission (by modulating the effects of adrenoceptor agonists), neural elements (by modulating the effects of preganglionic electrical stimulation), as well as ganglionic responses to nicotine. Besides monitoring pressor responses to sympathetic stimulation, we have also examined three other indices of sympathetic activity: heart rate and the contraction of two types of smooth muscle. This allowed us to compare, in several tissues from a single animal, the multiple and sometimes paradoxical effects of adenosine on sympathetic neurotransmission.

Methods

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA), weighing 250 to 350 g, were anesthesized with Nembutal (50–60 mg/kg i.p.); the left carotid artery was cannulated, allowing direct measurement of blood pressure and heart rate *via* a pressure transducer and cardiotachometer, and allowing periodic withdrawal of blood samples for assay of adenosine content. A triple catheter was also implanted in the right jugular vein; bolus injections of drugs (nicotine, phenylephrine and isoproterenol) could thereby be given without interrupting a continuous i.v. infusion of adenosine or its diluent (0.9% saline).

For electrical stimulation of preganglionic sympathetic nerves, rats were pithed by inserting a stainless-steel tube (13-gauge) through the orbit and the foramen magnum, into the spinal column to the level of the sixth cervical vertebra; this destroys the brainstem and effectively severs the spinal cord from the brain. Through this trocar, a steel rod (1 mm diameter) was inserted down the length of the spinal column, terminating in the sacral vertebrae. The portion of the rod that remained inside the trocar was insulated with a silastic sheath. The animal was respired artifically with room air (1 ml/100 g b.w., 50 strokes/min) *via* a tracheal cannula for the duration of the experiment. Skeletal muscle was paralyzed with pancuronium bromide (0.02 mg/kg i.v.) or *d*-tubocurarine (1.0 mg/kg i.v.), and an indifferent electrode was inserted under the skin of the right hindlimb. Monophasic pulses (0.1 msec duration, 2 pulses per sec, 30 V, 5 sec train duration) were applied with a Grass S11 stimulator.

Methods for measurement of arterial blood pressure, heart rate and smooth muscle contractions of the eyelid and vas deferens as well as procedures for determination of plasma adenosine concentrations are described in the preceding report (von Borstel *et al.*, 1986).

Drug treatments. Adenosine (5 mg/ml in 0.9% saline) was administered as a continuous i.v. infusion at rates of 10 to 100 μl/min, using a Harvard (Harvard Apparatus Co. Inc., South Natick, MA) syringe pump. Nicotine, PE, ISO and the four adenosine analogs, *R*-PIA, *S*-PIA and N-CPCA, generously provided by John W. Daly, and 2-ClA (Sigma Chemical Co., St Louis, MO) were administered as i.v. bolus injections in a volume of 0.1 ml of saline. A recovery time of at least 10 min was allowed to elapse between successive drug injections to ensure reproducibility of responses.

Statistics. Data were converted to percentage of control responses (*i.e.*, before administration of adenosine or adenosine analogs); these were expressed as means ± S.E. and compared by analysis of variance.

Results

Effect of adenosine infusion on sympathomimetic responses. The basal concentration of adenosine in arterial plasma was 1.54 ± 0.16 μM. Infusion of adenosine at rates ranging between 0.15 to 0.60 mg/kg/min increased arterial adenosine significantly ($P < .005$) to levels between 2.18 ± 0.36 and 4.10 ± 0.37 μM. The effects of these elevated adenosine levels on basal blood pressure, heart rate and smooth muscle tension are described in the preceding paper (von Borstel *et al.*, 1986). Elevation of circulating adenosine levels to 2 to 3 μM caused a 2- to 3-fold potentiation of responses to nicotine (40 μg/kg i.v.) by all four of the sympathomimetic indices examined (figs. 1–4). The magnitude of this potentiation decreased when arterial adenosine concentrations were increased beyond 4 μM.

Adenosine also modified sympathomimetic responses to ES and to adrenergic agonists; the magnitude and direction of its effect (inhibitory or potentiatory) varied among the end organs studied. Pressor responses to both ES and PE (5.0 μg/kg i.v.) were attenuated equally at all adenosine concentrations tested (fig. 1); responses to both stimuli were inhibited by more than 50% at adenosine concentrations of 4 μM. At the concentrations tested, adenosine had no effect on the contraction of eyelid muscle induced by PE (fig. 2) and caused an insignificant trend toward attenuation (maximum 30% inhibition at concentrations above 4 μM) of the contractile response to ES. Similarly, adenosine failed to modify significantly PE-evoked contractions of the vas deferens, even at concentrations exceeding 4 μM (fig. 3), but tended to reduce responses to ES. The

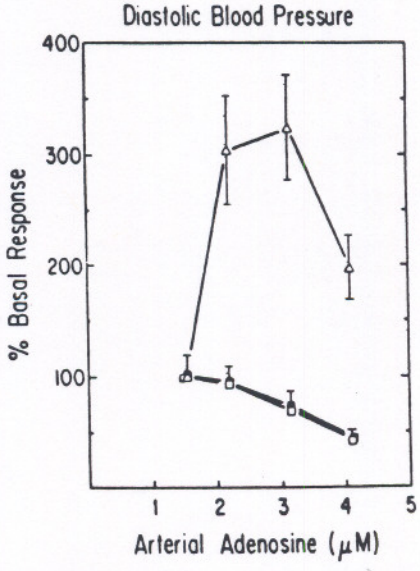

Fig. 1. Relationship between arterial plasma adenosine concentration and systemic pressor responses to nicotine (△, 40 μg/kg), phenylephrine (■, 5 μg/kg) and ES (□, 10–30V, 2 msec, 2 pps, 5 sec). Rats were tested before and during i.v. adenosine infusion at several controlled rates (0.15–0.5 mg/kg/min). Results are expressed as percentage of responses before adenosine infusion and reported as mean ± S.E.M. Control responses: nicotine, +25 ± 5 mm Hg; ES, +75 ± 4 mm Hg and PE, +49 ± 6 mm Hg from basal pressure of 67 ± 6 mm Hg. One-way analysis of variance indicates significant ($P < .01$) effect of arterial adenosine concentration on pressor responses to nicotine, PE and ES, $n = 6$.

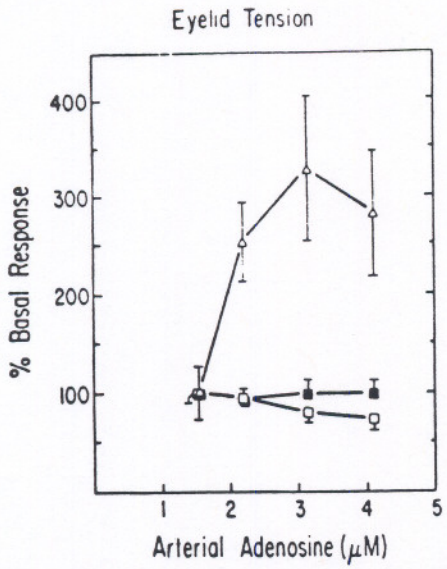

Fig. 2. Relationship between arterial plasma adenosine concentration and eyelid contractions in response to nicotine (△), PE (■) and ES (□). Results are expressed as percentage of responses before adenosine infusion and reported as mean ± S.E.M. Control responses: nicotine, +0.22 ± 0.05 g; ES, +0.57 ± 0.06 g; and PE, +0.21 ± 0.05 g. One-way analysis of variance indicates significant ($P < .01$) effect of adenosine on contractile responses to nicotine, $n = 6$.

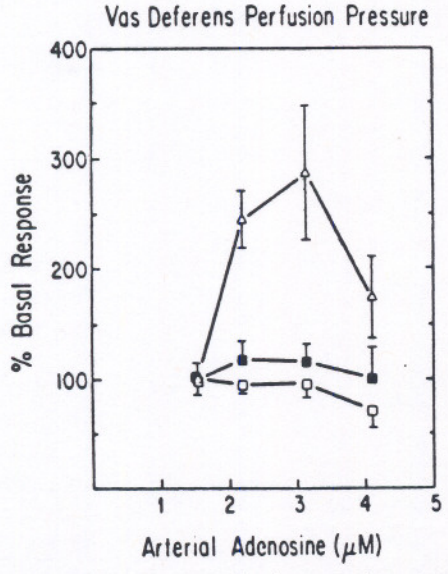

Fig. 3. Relationship between arterial plasma adenosine concentration and vas deferens contractions in response to nicotine (△), PE (■) and ES (□). Results are expressed as percentage of responses before adenosine infusion and reported as mean ± S.E.M. Control responses: nicotine, +11.2 ± 3.0 mm Hg; ES, +26.9 ± 3.7 mm Hg; and PE, +13.1 ± 2.0 mm Hg. One-way analysis of variance indicates significant ($P < .05$) effect of adenosine on response to nicotine. Two-way analysis of variance indicates significant ($P < .05$) difference between effects of adenosine on PE *vs.* ES, $n = 6$.

effects of adenosine on vas deferentia responses to ES *vs.* PE differed significantly ($P < .05$).

At the heart, adenosine failed to alter chronotropic responses to ES significantly (fig. 4), but did potentiate ISO-evoked (0.5 μg/kg i.v.) tachycardia by a maximum of 75% at adenosine concentrations of 3 μM. Inasmuch as adenosine concentrations above 2 μM tended to lower resting heart rates, it was possible that its enhancement of the chronotropic effect of ISO could have been due to lowered resting rates, increasing the potential spread between basal rates and the highest attainable "ceiling" heart rate. Therefore, the extent to which chronotropic responses to ISO and resting heart rates were correlated after adenosine administration was determined. Although a significant negative correlation was observed, it was relatively weak ($r^2 = 0.47$) accounting for less than half of the potentiation of ISOs chronotropic effect. Moreover, some animals displayed strong potentiation (80–100% increases) of ISO responses in the absence of large (>20 bpm) changes in resting heart rates. The effect of an i.v. infusion of nitroprusside (3–15 μg/kg/min), which produces vasodilation and bradycardia in pithed rats *via* a nonpurinergic mechanism, was also tested (table 1). Nitroprusside had no significant effect on ISOs chronotopic effect even though it lowered resting heart rates to the same extent as the adenosine infusion.

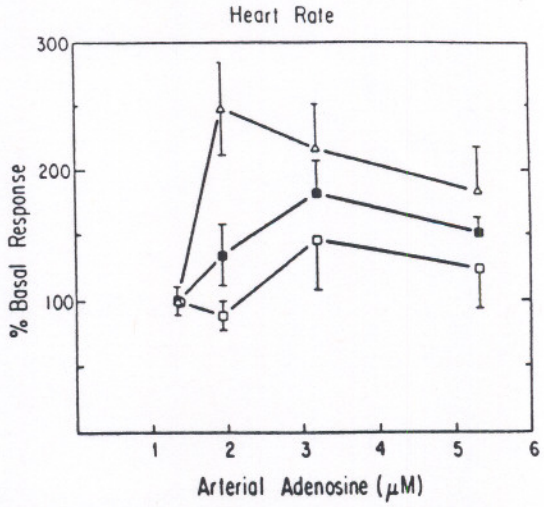

Fig. 4. Relationship between arterial plasma adenosine concentration and chronotropic responses to nicotine (△), ISO (■, 0.5 μg/kg) and ES (□). Results are expressed as percentage of responses before adenosine infusion and reported as mean ± S.E.M. Control responses: nicotine, +24 ± 2 bpm; ISO, +56 ± 6 bpm; and ES, +27 ± 4 bpm from basal rate of 329 ± 12 bpm. One-way analysis of variance indicates significant ($P < .05$) effect of adenosine on responses to nicotine and ISO, $n = 6$.

TABLE 1

Effect of nitroprusside infusion on heart rate responses to ISO

Control response to ISO (0.5 μg/kg i.v.), +79 ± 15 bpm.

	Nitroprusside infusion (μg/kg/min)			
	0.0	3.0	6.0	15.0
Basal heart rate (bpm)	334 ± 20	323 ± 19	304 ± 27	303 ± 25
Response to ISO (% of control)	100 ± 19	104 ± 10	122 ± 24	110 ± 16

Effects of adenosine analogs on sympathomimetic responses. Four synthetic adenosine analogs were also administered in an attempt to characterize the adenosine receptor subtype(s) involved in pre- or postsynaptic modifications of sympathomimetic responses. Previous studies indicated that an adenosine receptor resembling the A2 subtype mediates the potentiation by adenosine of sympathomimetic responses to nicotine at sympathetic ganglia (von Borstel *et al.*, 1984). The adenosine analogs N-CPCA, 2-CLA and the *R*- and *S*- diastereomers of PIA were administered i.v. in graded doses 1 min before challenge by ES, PE or ISO. Three of the four agonists suppressed to an equal extent pressor responses evoked by ES and PE (fig. 5); N-CPCA was most potent, exhibiting a threshold of action near 0.5 μg/kg and a half-maximal effect at 1 to 2 μg/kg; *R*-PIA was less potent, requiring doses of greater than 2.5 μg/kg to inhibit pressor responses significantly and doses of 5 to 10 μg/kg to produce 50% attenuation; 2-CLA was less

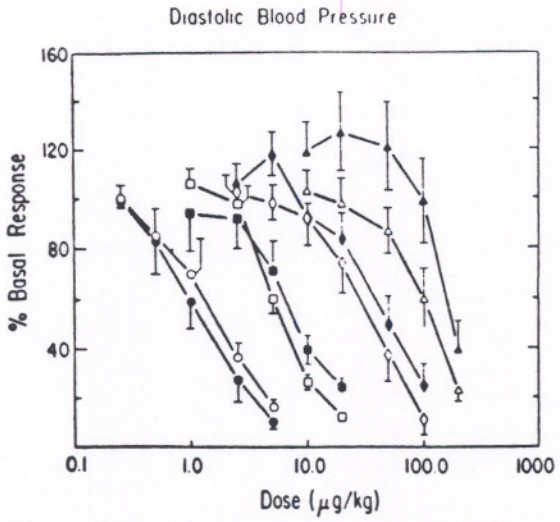

Fig. 5. Effect of adenosine analogs on systemic pressor responses to PE (closed symbols) and ES (open symbols). The adenosine derivatives N-CPCA (○), *R*-PIA (□), 2-CLA (◇) and *S*-PIA (△) were administered, in doses indicated on the abscissa, 1 min before challenge by PE or ES. Results are expressed as percentage of control responses and reported as mean ± S.E.M. Control responses: ES, +57 ± 4 mm Hg and PE, +47 ± 4 mm Hg from basal pressure of 52 ± 3 mm Hg. One-way analysis of variance indicates significant ($P < .001$) effect of N-CPCA, *R*-PIA, 2-CLA and *S*-PIA dose on pressor responses to both PE and ES, $n = 5$.

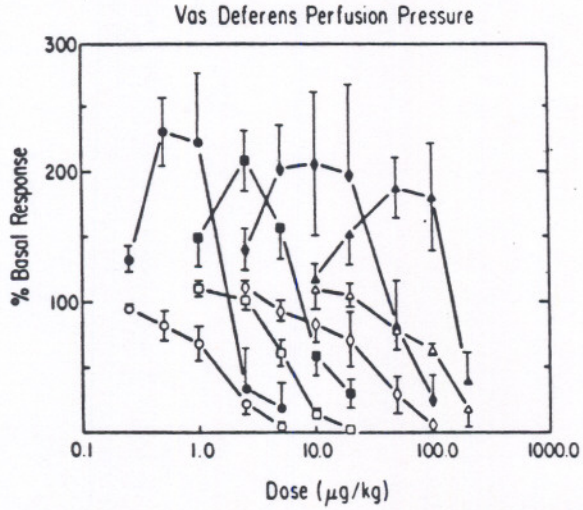

Fig. 6. Effect of adenosine analogs on vas deferens contractions in response to PE (closed symbols) and ES (open symbols). The adenosine derivatives N-CPCA (○), *R*-PIA (□), 2-CLA (◇) and *S*-PIA (△) were administered at indicated doses. Results are expressed as percentage of control responses and reported as mean ± S.E.M. Control responses: ES, +26 ± 2.3 mm Hg and PE, +10 ± 2 mm Hg. One-way analysis of variance indicates significant ($P < .05$ or better) effect of all drugs on both PE and ES responses except for *S*-PIA *vs.* PE. Two-way analysis of variance indicates significant difference between PE *vs.* ES for all four drugs, $n = 5$.

potent than *R*-PIA, with a threshold dose of approximately 10 μg/kg and a half-maximal effect at doses between 25 and 50 μg/kg. *S*-PIA was less than one-tenth as potent as *R*-PIA in attenuating pressor responses to ES; it potentiated pressor responses to PE slightly at doses less than 100 μg/kg, and suppressed them at doses greater than 100 μg/kg.

At the eyelid muscle, all four analogs reduced the contractions evoked by ES and PE, exhibiting a potency series similar to that observed for inhibition of pressor responses (N-CPCA > *R*-PIA > 2-CLA > *S*-PIA, data not shown). In contrast, at the vas deferens, low doses of adenosine analogs significantly potentiated PE-evoked contractions, whereas higher doses suppressed them (fig. 6). Responses to ES were not potentiated, but instead were attenuated by all four drugs at doses which potentiated the effects of PE; thus, significant differences ($P < .05$) were observed between the actions of each drug on PE- *vs.* ES-evoked contractions. The rank order of potency N-CPCA > *R*-PIA > 2-CLA > *S*-PIA was observed for effects on responses to both ES and PE.

The same adenosine analogs were also tested for their ability to alter changes in heart rate evoked by ISO and ES. N-CPCA potentiated the chronotropic effects of ISO and ES (figs. 7 and 8); the latter effect was abolished as the dose of N-CPCA was increased beyond 2.5 μg/kg, converting to inhibition of responses to ES. Of the other three drugs, only *R*-PIA had a significant effect on ISOs chronotropic effect, attenuating the response to ISO by 50% at a dose of 50 μg/kg; all three

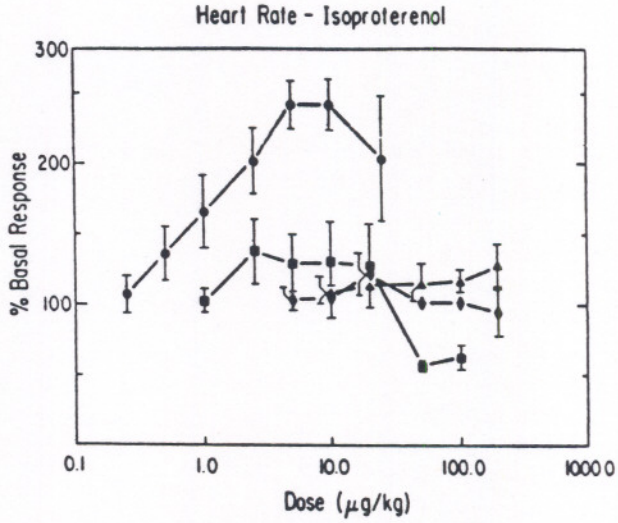

Fig. 7. Effect of adenosine analogs on chronotropic responses to isoproterenol. The adenosine derivatives N-CPCA (●), *R*-PIA (■), 2-CLA (♦) and *S*-PIA (▲) were administered at indicated doses. Results are expressed as percentage of control responses and reported as mean ± S.E.M. Control responses: ISO, +80 ± 10 bpm from basal rate of 347 ± 11 bpm. One-way analysis of variance indicates significant ($P < .05$) effect of N-CPCA and *R*-PIA on responses to ISO, $n = 5$.

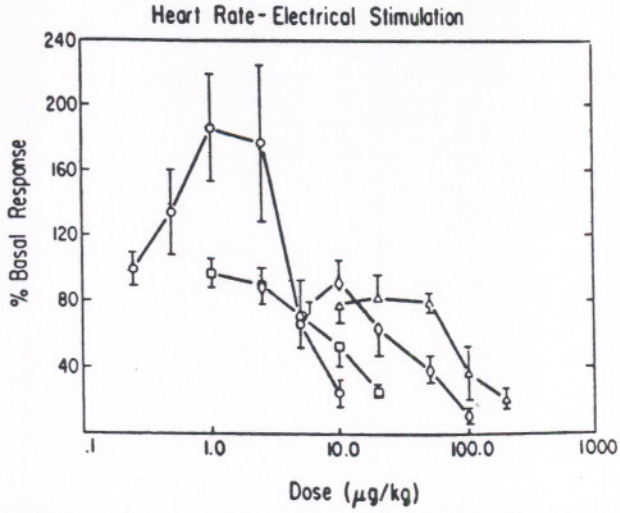

Fig. 8. Effect of adenosine analogs on chronotropic responses to ES The adenosine derivatives N-CPCA (○), *R*-PIA (□), 2-CLA (◇) and *S*-PIA (△) were administered at indicated doses. Results are expressed as percentage of control responses and reported as mean ± S.E.M. Control responses: ES, +42 ± 7 bpm from basal rate of 347 ± 11 bpm. One-way analysis of variance indicates significant ($P < .05$) effect of all drugs on responses to ES, $n = 5$.

significantly reduced the chronotropic response to ES, exhibiting the potency series *R*-PIA > 2-CLA > *S*-PIA.

Discussion

Our data demonstrate that circulating adenosine and its synthetic congeners can elicit multiple effects on the efferent components of the sympathetic nervous system. Adenosine may produce the changes observed here by acting at sympathetic ganglia, noradrenergic nerve terminals and/or sympathetically innervated end organs. At ganglia and end organs, adenosine may either facilitate or inhibit neurotransmission (von Borstel *et al.*, 1984; Henon and McAfee, 1983; Hedqvist *et al.*, 1978; Clanachan *et al.*, 1977), whereas at noradrenergic nerve terminals, adenosine has been shown to attenuate neurotransmitter release (Wakade and Wakade, 1978; Fredholm and Hedqvist, 1980; Hedqvist *et al.*, 1978). Effects of the adrenergic agonists PE and ISO are mediated primarily by adrenoceptors located on sympathetically innervated end organs (Gilman *et al.*, 1980), providing an index of the direct effect of adenosine on tissue responsiveness to adrenergic stimulation. Responses to ES involve, in addition, pre- and postganglionic neural elements. Therefore, differences between the effects of adenosine on responses to ES and PE reflect relative contributions of neural *vs.* end organ processes to the net influence of adenosine on sympathetic transmission.

Adenosine potentiated the sympathomimetic effects of nicotine at concentrations (2–3 μM) which produced only modest (less than 25%) inhibition of responses to ES and PE.. At these lower concentrations it is apparent that adenosine's potentiatory effect, presumably mediated through ganglionic adenosine receptors (von Borstel *et al.*, 1984), can override its well-documented inhibitory influence at the sympathetic neuroeffector junction (Lokhandwala, 1979; Sollevi *et al.*, 1981). Thus, potentiation of nicotine's ganglionic effects appears to be adenosine's most potent action *in vivo*. As adenosine levels are elevated to and beyond 4 μM, its inhibitory action on responses to ES and PE becomes significant, coinciding with a reduction in the potentiation of nicotine's effect.

The relative contributions of pre- and postsynaptic inhibition by adenosine appear to vary from tissue to tissue. Equal inhibition of the systemic pressor responses to ES and PE suggests that postsynaptic mechanisms may be sufficient to account for adenosine's inhibition of vascular sympathetic transmission during ES. However, this does not preclude an additional presynaptic contribution to reduction of pressor response to ES, as has been demonstrated in isolated rat vascular tissue *in vitro* (Kamikawa *et al.*, 1980; Kubo and Su, 1983; Enero and Saidman, 1977). The potency series N-CPCA > *R*-PIA > 2-CLA > *S*-PIA is similar to that observed for inhibition of pressor responses to noradrenergic stimulation in the isolated guinea-pig aorta (Collis and Brown, 1983).

At the vas deferens, unlike vascular and eyelid smooth muscle, the effect of adenosine analogs on PE-induced contraction was biphasic; responses to PE were potentiated at low doses and inhibited at higher doses (fig. 6). This could indicate the presence of two or more subpopulations of postsynaptic adenosine receptors, differing in both their affinities for adenosine and the direction of their effects on vas deferens responses to adrenergic stimuli. Although enhancement of postsynaptic responses to adrenergic agonists has not been reported previously in the rat (Clanachan *et al.*, 1977), this phenomenon has been detected in the isolated guinea-pig vas deferens (Holck and Marks, 1978).

Adenosine receptor subtypes have been defined according to several characteristics. The A1 (or Ri) subtype, as characterized in the central nervous system, inhibits adenylate cyclase, has a high affinity constant (10–100 nM) for adenosine and is characterized by the potency series *R*-PIA > 2-CLA, N-CPCA > *S*-PIA; the A2 (or Ra) subtype activates adenylate cyclase, has a lower affinity for adenosine (2–20 μM) and is characterized by the potency series N-CPCA, D-N-ethylcarboxamido adenosine > 2-CLA > *R*-PIA > *S*-PIA (Van Calker *et al.*, 1979, Daly *et al.*, 1981; Londos *et al.*, 1980). This classification scheme may not be generally applicable to all adenosine receptors; many actions of adenosine do not appear to be mediated through alterations in adenylate cyclase activity and peripheral receptors have not yet been defined rigorously according to the criteria established for central adenosine receptors.

Adenosine analogs potentiate sympathomimetic responses to nicotine with a potency order of N-CPCA > 2-CLA > *R*-PIA > *S*-PIA, suggesting the involvement of A2 receptors (von Borstel *et al.*, 1984; see also preceding report). However, the rank order of potency for attenuation of pressor effects of ES and PE, and for both potentiation of responses to PE and attenuation of responses to ES in the vas deferens was N-CPCA > *R*-PIA > 2-CLA > *S*-PIA, and 2- to 10-fold higher doses of the analogs were needed to produce these effects as compared to doses needed to potentiate responses to nicotine. This latter order does not conform clearly to the A1 *vs.* A2 classification (which is now based solely on relative potencies of adenosine agonists), suggesting either differences in receptor subtypes mediating adenosine's effects at ganglia *vs.* neuroeffector junctions or differences in drug bioavailability at the two sites.

Adenosine's potentiation of chronotropic responses to ISO was unexpected; *in vitro*, adenosine reduces cardiac norepinephrine release and attenuates the inotropic and chronotropic effects of *beta* adrenergic stimulation (Rockoff and Dobson, 1980; Rardon and Bailey, 1984; Dobson, 1980). In the present *in vivo* studies, circulating adenosine concentrations of 3 μM or more potentiated the positive chronotropic effect of ISO; in addition, N-CPCA potentiated the effects of both ISO and ES. N-CPCA is more potent as a coronary vasodilator than the other analogs tested in this study (Evans *et al.*, 1982); consequently, improved myocardial access of circulating catecholamines might explain N-CPCAs enhancement of the positive chronotropic effects of ISO and ES and could underlie the potentiation of ISO effects produced by adenosine itself *in vivo* (Seitelberger *et al.*, 1984) (fig. 4). However, the failure of the nonpurinergic vasodilator nitroprusside to produce similar potentiation of ISOs chronotropic effect suggests that the potentiation was produced by a more specific purinergic mechanism which was not secondary to systemic or coronary vasodilation.

The action of adenosine in potentiating responses to nicotine but not to ES was somewhat surprising, as both are generally presumed to involve stimulation of nicotinic ganglionic receptors. The present finding contributes to the body of evidence suggesting that different subsets of nicotine receptors may mediate the effects of endogenous acetylcholine and of exogenous nicotinic agonists. Thus, at ganglia, autodesensitization to nicotine does not impair postganglionic responses to ES (Bentley, 1972), and the hemicholinium analog α,α′-bis(dimethylammoniumacetaldehyde diethylacetal)*p,p′*-diace-

tylphenyldibromide blocks ganglionic stimulation by nicotine, tetramethylammonium or dimethylpiperazinium without blocking the effects of ES (Wong and Long, 1968). We have found that several nicotinic antagonists, including *d*-tubocurarine, can block sympathomimetic effects of nicotine (in the presence or absence of adenosine) at doses which have little effect on responses to ES (unpublished data). Adenosine potentiates sympathomimetic responses to other nicotinic agonists including lobeline, dimethylphenylpiperazinium iodide, sebacyl choline and tetramethylammonium chloride, indicating its interaction with nicotine reflects a general effect, one not peculiar to nicotine itself (von Borstel *et al.*, 1986).

In summary, these data indicate that adenosine modulates sympathetic neurotransmission through actions at multiple sites including ganglia, presynaptic noradrenergic nerve terminals and postsynaptic end organs receiving sympathetic innervation. The net effect of an increase in circulating adenosine levels on sympathetic transmission reflects the aggregate of its individual actions at these anatomical loci, as well as the possible existence of different subpopulations of adenosine receptors within some of these sites. The multiplicity of adenosine's effects within the sympathetic nervous system suggests that the nucleoside and its congeners, as well as compounds that inhibit their actions (like caffeine), may possess a much more subtle array of effects than are currently attributed to them.

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