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Presynaptic actions of piribedil on the cardiovascular system of the pithed rat

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The influence of piribedil on cardiovascular sympathetic responses has been studied in the pithed rat. Piribedil (0·3–1 mg kg^{-1}) inhibited the increases of diastolic blood pressure induced by spinal cord electrical stimulation at the level Th5-L4. This effect was reversed by sulpiride (0·3 mg kg^{-1}) but not by yohimbine (0·3 mg kg^{-1}). The cardiovascular responses induced by noradrenaline were unaffected by piribedil (0·3–1 mg kg^{-1}). However piribedil (0·3–1 mg kg^{-1}) did not modify the heart rate increase induced by spinal cord electrical stimulation at the C7-Th1 level. These results suggest that piribedil inhibits the vascular sympathetic transmission in the pithed rat via stimulation of presynaptic dopamine receptors.

The existence of peripheral presynaptic dopamine receptors in addition to presynaptic α_2-adrenoceptors has been accepted and their activation also induces an inhibition of neurotransmitter release during sympathetic nerve stimulation. These presynaptic dopamine receptors seem to be different from those located at the postsynaptic level (Langer 1981; Cavero et al 1982a, b). Piribedil is a piperonyl-pirimidil derivative known as a central dopamine receptor agonist (Corrodi et al 1972). This compound has been used clinically in the treatment of extrapyramidal (Fuxe 1973; Goldstein et al 1973; Truelle et al 1977), as well as in neuroendocrine disturbances due to a dysfunction of the central dopaminergic system (McLeod & Lehmeyer 1974; Camanni et al 1975). This drug also acts on the peripheral nervous system. It inhibits sympathetic neurotransmission to femoral, splenic, mesenteric, cardiac and renal arteries of the dog (Laubie et al 1977; Laubie & Schmitt 1978), nictitating membrane of the cat (György & Doda 1981), and in isolated kidney of the rabbit (Chevillard et al 1980), effects that have been attributed to an action on prejunctional inhibitory dopamine receptors. It has also been reported that many agonists acting on dopamine receptors have mixed α-adrenoceptor/dopamine effects (Lokhandwala & Barret 1982). Several studies have reported that the pithed rat is a suitable model to study drugs acting at both presynaptic and postsynaptic α-adrenoceptors and presynaptic dopamine receptors in the cardiovascular system (Clapham & Hamilton 1982; Wilffert et al 1984; Vila et al 1985). The purpose of the present study has been to evaluate the action of piribedil on the different catecholamine receptors that participate in the sympathetic vascular and cardiac responses in the pithed rat.

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Materials and methods

Male, Sprague-Dawley rats (290–320 g) received atropine (1 mg kg^{-1}, i.p.) and 15 min later were anaesthetized with pentobarbitone sodium (75 mg kg^{-1} i.p.). The animals were pithed as described by Gillespie et al (1970). The trachea was cannulated and the animals were ventilated with room air (60 strokes min^{-1}, 1 ml/100 g body wt). The right jugular vein was cannulated for drug injections. Arterial blood pressure was measured using a Statham pressure transducer connected to a cannula placed in the right carotid artery. Heart rate was derived from the arterial pulse and both parameters were displayed on a Hewlett Packard (mod 7785A) recorder. Bilateral adrenalectomy and vagotomy were then performed. Increases in diastolic blood pressure (DBP) were elicited by electrical stimulation (0·25–6 Hz, 60 V, 0·5 ms) of the entire sympathetic outflow and bolus injection of noradrenaline (0·01 to 3·0 μg kg^{-1} i.v.). Increases of heart rate (HR) were obtained by stimulation at the level C7-Th1 (0·25–6 Hz, 0·5 ms, supramaximal voltage). When rats were electrically stimulated, they received (+)-tubocurarine (1 mg kg^{-1}, i.v.). Except for the HR studies all animals received propranolol (1 mg kg^{-1}). Results are expressed as mean ± s.e. of the mean for groups of identical experiments. Statistical comparisons were made by Student's *t*-test.

Results

Electrical stimulation of the spinal cord gives rise to a frequency-dependent increase on DBP (Fig. 1A). Bolus injection of piribedil (0·3 and 1 mg kg^{-1}) did not modify the basal DBP of pithed normotensive rats (39 ± 1·35 mmHg, n = 12) but produced a significant dose-dependent inhibition of DBP induced by electrical stimulation at low frequencies (Fig. 1A). The same doses of piribedil did not modify the dose-dependent DBP increases induced by administrtion of noradrenaline (0·01 to 3·0 μg kg^{-1}) (Fig. 1B).

Pretreatment with sulpiride (0·3 mg kg^{-1}), administered 5 min before, abolished the piribedil (0·3–1 mg kg^{-1}) inhibition on the stimulation-induced increases of DBP (Fig. 2A). The frequency-responses curves of the increase on DBP were slightly increased by pretreatment with yohimbine (0·3 mg kg^{-1}) (Fig. 2B). This difference was not statistically significant when compared with the control curve (Fig. 1A). Pretreatment with yohimbine (0·3 mg kg^{-1}) did not antagonize the inhibition of DBP increases induced by piribedil (0·3 and 1 mg kg^{-1}) (Fig. 2B).

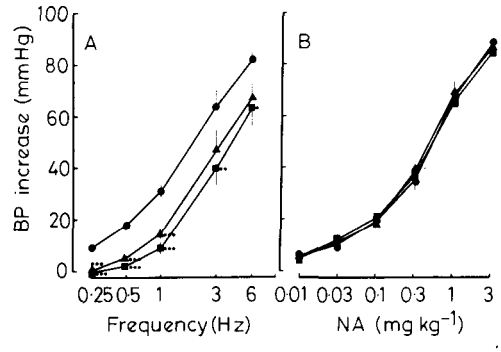

FIG. 1. Effects of piribedil on increases of diastolic blood pressure elicited by electrical stimulation of the spinal cord (A) and noradrenaline (B) in the pithed rat after pretreatment with saline (●—●); piribedil 0·3 mg kg^{-1} (▲—▲); piribedil 1 mg kg^{-1} (■—■). Each point is the mean of 6–8 experiments. Vertical bars show standard error of the mean.

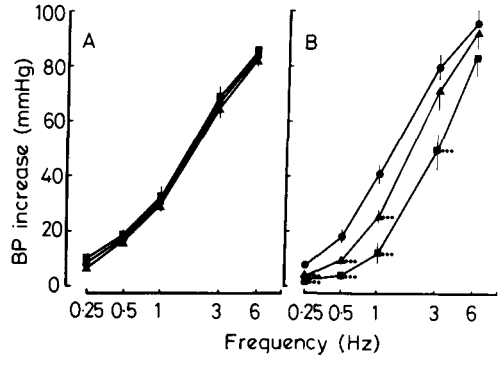

FIG. 2. Stimulation-induced increases of diastolic blood pressure in the pithed rat after pretreatment with saline (●—●); piribedil 0·3 mg kg^{-1} (▲—▲); piribedil 1 mg kg^{-1} ■—■, given in presence of sulpiride 0·3 mg kg^{-1} (A); yohimbine 0·3 mg kg^{-1} (B). Each point is the mean of 6 experiments. Vertical bars show standard error of the mean.

The resting HR of pithed normotensive rats was 308·1 ± 11·7 beats min^{-1} (mean ± s.e., n = 21). A bolus injection of piribedil (0·3 and 1 mg kg^{-1}) did not modify the basal HR. Electrical stimulation at the level C7-Th1 of the spinal cord induced a frequency–dependent increase in HR. Piribedil (0·3 and 1 mg kg^{-1}) did not modify the frequency-response curve of HR increases (results not shown).

Discussion

Our results demonstrate that piribedil inhibits the stimulation-induced pressor responses in the pithed rat via activation of presynaptic dopamine receptors. This inhibitory action of piribedil was prevented by previous administration of a dopamine receptor antagonist such as sulpiride. Some evidence exists that sulpiride antagonizes α_2-adrenoceptors in the rat vas deferens (Badia et al 1982), however, the doses used in this study are devoid of α_2-adrenoceptor blocking properties (Vila et al 1985). In addition, the classical α_2-adrenoceptor blocking drug yohimbine did not influence the inhibitory effects of piribedil and hence an action of piribedil on α_2-adrenoceptors can be ruled out. On the other hand, piribedil did not modify the vasopressor responses induced by noradrenaline and the possibility of an action of piribedil on vascular postsynaptic α-adrenoceptors is not supported. These results do not agree with the finding that piribedil reduces vasoconstrictor effects of noradrenaline in the isolated kidney of the rabbit (Chevillard et al 1980). In contrast, piribedil did not impair vasoconstrictor effects of noradrenaline in the dog (Laubie & Schmitt 1978) nor the nictitating membrane contraction by noradrenaline in the cat (György & Doda 1981), results that confirm our findings. Piribedil did not inhibit the stimulation-induced tachycardia in the pithed rat. This observation demonstrates a lack of action of piribedil on presynaptic α_2-adrenoceptors as it is known that at the cardiac sympathetic nerve terminals of the rat, prejunctional α_2-adrenoceptors, but not dopamine receptors, are present (Cavero et al 1981; Clapham & Hamilton 1982; Wilffert et al 1984; Vila et al 1985).

In conclusion, our findings show that piribedil inhibits the vascular sympathetic transmission in the pithed rat through a reduction of noradrenaline release by stimulation of presynaptic dopamine receptors. However, the present study does not allow us to elucidate whether these dopamine receptors are localized in the sympathetic ganglia and/or on postganglionic sympathetic nerve terminals. This action on presynaptic dopamine receptors can participate in the vasodilator effect of piribedil (Buylaert 1977; Laubie et al 1977). Finally piribedil, in contrast to many other dopamine receptor agonists (Hamed et al 1981; Clapham & Hamilton 1982; Wilffert et al 1984; Vila et al 1985), did not show effects on α_2-adrenoceptors.

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Enhanced serum concentrations of Ara-C using suppositories containing tetrahydrouridine as a deamination inhibitor of Ara-C

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Rectal bioavailability of Ara-C (serum AUC 4 h: 65 μg h^{-1} ml^{-1}) administered in a suppository formulation containing tetrahydrouridine (a deamination inhibitor) and sodium salicylate (an adjuvant) to dogs was better than that from a suppository formulation without tetrahydrouridine (serum AUC 4 h: 18 μg h^{-1} ml^{-1}).

Ara-C (1-β-D-arabinofuranosylcytosine) has been administered by slow intravenous infusions for the treatment of lymphatic cancers (Goodell et al 1970; Bickers et al 1974) due to its poor absorption in the gastrointestinal tract (Wan et al 1974). We have reported (Nishihata et al 1986) that, on rectal administration, Ara-C in a microenema containing either the adjuvants 5-methoxysalicylate or glycerol monooleate–glycerol (1 : 1 v/v) in rats shows preferential absorption into the lymphatic system. Thus, rectal administration provides a non-invasive method of targeting the drug to the site of action with a consequent reduction in systemic side effects.

Here we report the effects of suppositories containing the adjuvants sodium salicylate (300 mg) with or without 1(-β-D-ribofuranosyl)-4-hydroxy tetrahydro-2[1*H*]-pyrimidine, (3,4,5,6-tetrahydrouridine, THU) on the serum levels of Ara-C and its major metabolite Ara-U (1-β-D-arabinofuranosyluracil) in adult beagle dogs.

The deamination of Ara-C to a biologically inactive product Ara-U by pyrimidine nucleoside deaminase (cytidine aminohydrolase) has been studied both in-vitro (Camiener 1967a, b; Camiener & Smith 1965; Loo et al 1965) and in-vivo (Camiener 1968; Dedrick et al 1973). Ho & Frei (1971) and Wan et al (1974) have reported that the disappearance from plasma of Ara-C was biphasic with a short-life of 11 min and a longer half-life of 111 min. They also demonstrated dose-related pharmacokinetics which indicated that the deamination of Ara-C was a saturable process. The deamination process can be partially inhibited by THU (Camiener 1968). Furthermore, it has been reported by Camiener (1968) that THU shows no noticeable toxic effects in rats, mice, monkeys and dogs. Thus, we would expect to see elevated serum Ara-C levels and reduced Ara-U levels from suppositories containing THU. However, such observations were not seen.

Methods

Suppository preparation. Ara-C (Upjohn), 33 mg, and where appropriate THU (Sigma), 1 mg, were dissolved in 260 μl distilled water. To the solution was added molten Witepsol S55 (Dynamit Nobel), 700 mg, with thorough mixing. To the mixture was added sodium salicylate (99% + Aldrich), 300 mg, in 25 mg amounts each of which was thoroughly dispersed in the mixture before the next addition. The molten mass was then cooled with stirring until just above the solidification point and poured into a 1 g suppository mould and the product stored for 24 h before administration. For i.v. injections, 30 mg of Ara-C was dissolved in 1 ml of distilled water.

Five normal, healthy beagle dogs, 10·6 to 14·5 kg were used with a period of one week between successive drug administrations. The animals were fasted with free access to water for 24 h before use. Suppositories were inserted to a depth of 4 cm from the outer rectal sphincter, and blood samples were taken from the cephalic vein at the following times: 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min. Blood was withdrawn at the same times after intravenous administration of Ara-C; the i.v. injection was administered in the cephalic vein

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