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The influence of α_1- and α_2-adrenoceptor agonists on cardiac output in rats and cats

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In pithed rats, but not in pithed cats, the α_2-/dopamine agonist B-HT 920 (10–300 μg kg^{-1}) increased cardiac output. The increase was inhibited by yohimbine (1 mg kg^{-1}), but not by sulpiride (0·3 mg kg^{-1}) and nifedipine (1 mg kg^{-1}). Both in pithed rats and cats, the α_1-adrenoceptor agonist methoxamine (30–300 μg kg^{-1} and 30–1000 μg kg^{-1}, respectively) increased cardiac output. This increase (rats) was inhibited by prazosin (0·1 mg kg^{-1}). The results indicate the existence of functional venous α_2-adrenoceptors in pithed rats, and their absence in pithed cats. Furthermore, α_2-adrenoceptor-mediated venoconstriction appears independent of extracellular calcium.

Experiments with ergotamine in pithed rats (Kalkman 1983) have provided indications for the presence of functional α_2-adrenoceptors at the venous site. We have sought confirmation for this observation by application of the selective α_2-adrenoceptor agonist B-HT 920. Very recently, Gerold & Haeusler (1983) reported cardiac output increases of pithed rats to a single dose of the α_2-adrenoceptor agonists, clonidine and B-HT 920. If we assume that in the pithed rat preparation an increase in cardiac output reflects venous constriction, that is when heart rate remains constant, the observations by Gerold & Haeusler (1983) indeed suggest the presence of α_2-adrenoceptors located in the venous vascular bed.

Apart from its agonistic activity on α_2-adrenoceptors, B-HT 920 has also been demonstrated to possess stimulatory activity at central and peripheral dopaminergic receptors (Andén et al 1982; Wilffert et al 1984). We therefore extended our investigation with selective antagonists of α_2-adrenoceptors (yohimbine) and dopamine receptors (sulpiride), respectively.

Zandberg et al (1984) have shown the absence of functional venous α_2-adrenoceptors in ganglion-blocked spinalized dogs. For this reason we also investigated the cardiac output response to B-HT 920 in pithed cats, in order to obtain information on this question in another animal species. For reasons of comparison the cardiac output effects after α_1-adrenoceptor stimulation (methoxamine) were investigated as well.

Materials and methods

Rats. Male Wistar normotensive rats (300–350 g) were pithed under hexobarbitone anaesthesia (150 mg kg^{-1} i.p.), and subjected to artificial respiration with room air. Rectal temperature was maintained at approximately 37 °C. Blood pressure was recorded from the left carotid artery on a Hellige HE-19 device. Drugs and 0·9% NaCl (saline) were injected i.v. via the cannulated right jugular vein. Heart rate was derived from the arterial pulse-wave (HSE EKA-PULSE). Cardiac output was determined by thermodilution-technique as described by Fegler (1954). A thermistor of 100 k ohms (Philips) was mounted on a PE-50 catheter and incorporated into a Wheatstone bridge. The PE-50 catheter was introduced into the aorta via the right carotid artery. Saline solution (0·1 ml, 20–24 °C) was rapidly injected into the right jugular vein. The change in temperature caused by the saline injection, measured by the thermistor, was displayed on a Mettler CG 20 recorder. Computations of the cardiac output were performed by means of the Hamilton-Stewart formula (Fegler 1954) on a Hewlett-Packard 41 C Device. Values were expressed as ml/100 g weight. The average of three successive cardiac index measurements was taken as 100%. Alterations from this 100%-value, provoked by injection of the agonist, were evaluated by a paired *t*-test. Each rat received a single dose of an agonist. Each dose was given to 6–8 rats. The cardiac index was determined at 1, 3, 5 and 10 min after injection of the agonist.

Cats. Mongrel cats of either sex (2·1–2·5 kg) were pithed under chloralose anaesthesia (60 mg kg^{-1} i.p.) and subjected to artificial respiration. Left-side thoracotomy was performed by severing the first four ribs from the sternum and subsequently the ascending aorta was exposed. Cardiac output was estimated by measurement of ascending aortic blood flow using an electromagnetic flow transducer (Transflow 601, Skalar, Delft, The Netherlands) placed around the proximal part of the aortic arch and connected to a flow meter (Skalar 503 System, Delft, The Netherlands). The flow-probe signal was electronically differentiated in order to obtain dø/dt values, serving as a rough indication for cardiac contractility. The stroke-volume was constantly monitored by electronic integration of the flow-probe signal. Blood pressure was recorded from the left femoral artery via a Statham P23 Db pressure transducer. All variables were recorded continuously on a Hellige HE-21 polygraph. The agonists, B-HT 920 and methoxamine, were injected via a cannulated femoral vein in a cumulative way. Cardiac output values were

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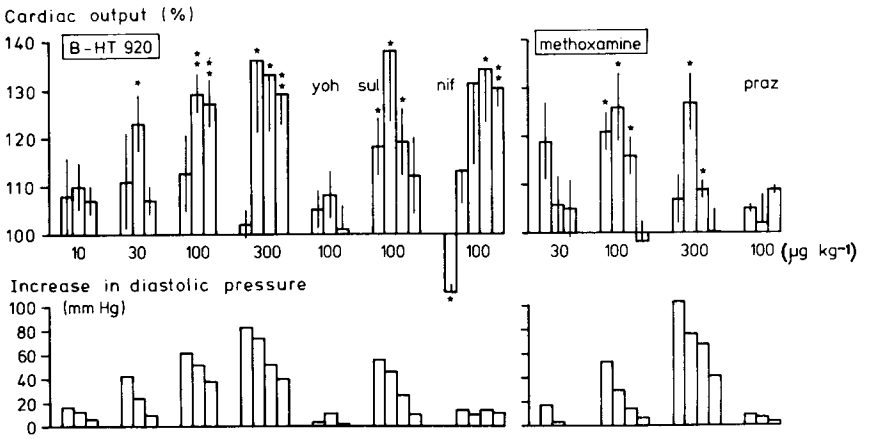

FIG. 1. Increase in cardiac output (ml min^{-1}/100 g; mean ± s.e.m.) of pithed normotensive rats by B-HT 920 and methoxamine (upper half). Cardiac output measured by the thermodilution technique was estimated at 1, 3, 5, and eventually 10 min after administration of a single dose of the agonist. The alterations with respect to the control value of the individual animal (= 100%) were evaluated for significance in a paired *t*-test (* $P < 0.05$; ** $P < 0.01$). Each dose was measured in at least 6 animals. In the lower half of the figure the mean values of the maximal increase of diastolic blood pressure, elicited by the various doses of the agonists, are presented. Note that the maximal pressure increase took place earlier than the maximal cardiac output. The effects of pretreatment with yohimbine (yoh, 1 mg kg^{-1}), sulpiride (sul, 0·3 mg kg^{-1}), nifedipine (nif, 1 mg kg^{-1}) and prazosin (praz, 0·1 mg kg^{-1}) on both parameters are shown as well.

corrected for body weight and expressed as ml min^{-1} kg^{-1}.

Drugs. B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-[5,4-d]-azepine.2HCl, Thomae) and methoxamine HCl (Burroughs Wellcome) were dissolved in saline. Yohimbine HCl (Sigma) and prazosin HCl (Pfizer) were dissolved in a 10% w/v glucose solution. Nifedipine (Bayer) was dissolved in 96% ethanol (final concentration 1%), and diluted with glucose solution (10% w/v) to obtain a concentration of 1 mg ml^{-1}. Sulpiride (Delagrange) was dissolved in a 0·02 M tartaric acid solution.

Results

Rats. Both methoxamine (30–300 μg kg^{-1}) and B-HT 920 (10–300 μg kg^{-1}) dose-dependently increased blood pressure and cardiac output. The maximal value of cardiac output was reached at a later time than that of the blood pressure increase (Fig. 1). Heart rate remained unchanged. The initial value (mean ± s.e.m.) of the cardiac output (ml min^{-1}/100 g) of the different subgroups (n = 6–8) amounted to 22·3 ± 1·9, 26·7 ± 1·6 and 25·2 ± 1·9 for 30, 100 and 300 μg kg^{-1} of methoxamine, respectively; 23·2 ± 1·7 for methoxamine (100 μg kg^{-1}) after prazosin; 22·9 ± 0·9, 23·6 ± 1·2, 25·2 ± 1·7 and 20·5 ± 1·6 for 10, 30, 100 and 300 μg kg^{-1} of B-HT 920, respectively; 21·2 ± 1·3 for B-HT 920 (100 μg kg^{-1}) after yohimbine; 17·8 ± 1·4 after sulpiride and 24·7 ± 1·0 after nifedipine. The relative increases in cardiac output were evaluated by a paired *t*-test and are shown in Fig. 1.

The antagonists, prazosin (0·1 mg kg^{-1}), yohimbine (1 mg kg^{-1}) and sulpiride (0·3 mg kg^{-1}) did not alter basal cardiac output. Nifedipine (1 mg kg^{-1}), but not its vehicle, caused a significant increase in cardiac output ($P < 0.05$; paired *t*-test). Before nifedipine, the mean cardiac output in this group of rats amounted to 21·7 ± 0·6 ml min^{-1}/100 g (n = 7).

The increase in cardiac output by B-HT 920 (100 μg kg^{-1}) was neither inhibited by sulpiride nor by nifedipine (Fig. 1). Pretreatment with yohimbine prevented the rise in cardiac output after B-HT 920 (Fig. 1). Prazosin (0·1 mg kg^{-1}) inhibited the increase in cardiac output after methoxamine (100 μg kg^{-1}).

Cats. Methoxamine (30–100 μg kg^{-1}) dose-dependently increased cardiac output and blood pressure (Fig. 2), but did not alter dø/dt. The initial value of cardiac output amounted to 67·4 ± 5·4 ml min^{-1} kg^{-1} for the cats receiving B-HT 920 and to 66·2 ± 3·4 for those of the methoxamine group. B-HT 920 (10–1000 μg kg^{-1}) elevated blood pressure, but left cardiac output and dø/dt unaltered (Fig. 2). Both agonists did not change resting heart rate. As observed in rats, the maximal value of the cardiac output increase induced by methoxamine was obtained later than that of the blood pressure rise.

Discussion

In pithed rats, the selective α_1-adrenoceptor agonist methoxamine, as well as the selective α_2-adrenoceptor agonist B-HT 920, dose-dependently increased the cardiac output and peripheral resistance. In pithed animal preparations lacking any CNS regulatory functions, an acute increase in cardiac output can be the result of an increased heart rate, an increase in cardiac contractile force, an increase in venous return (by

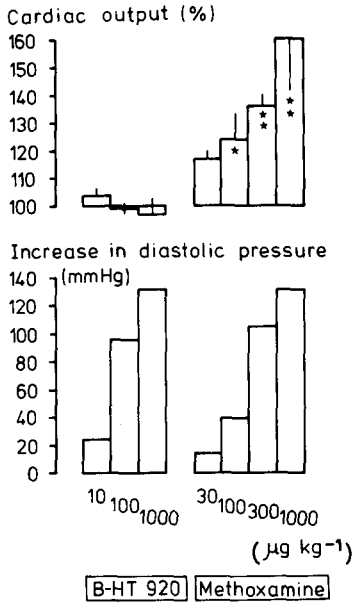

FIG. 2. Peak changes in resting cardiac output (= 100%) and diastolic blood pressure (mm Hg) due to cumulative doses of the α-adrenoceptor agonists B-HT 920 (10–1000 μg kg^{-1}) or methoxamine (30–1000 μg kg^{-1}) in pithed cats. Initial values of cardiac output amounted to 67·4 ± 5·4 and 66·2 ± 3·4 ml min^{-1} kg^{-1} (means ± s.e.m., n = 4) for cats receiving B-HT 920 and methoxamine, respectively. Results were evaluated for significance by a paired *t*-test; * $P < 0.05$; ** $P < 0.01$. Note that B-HT 920 increased blood pressure only.

venous constriction or by arteriolar dilatation) or by a combination of these factors (Guyton 1976). In the present study, methoxamine nor B-HT 920 induced changes in cardiac frequency in pithed rats. Cardiac α_1-adrenoceptors may mediate positive inotropism in rats (Schümann 1980) but no evidence exists for the presence of cardiac α_2-adrenoceptors mediating an increase in cardiac contractility. It is therefore unlikely, that B-HT 920 increases cardiac output by positive inotropism after stimulation of cardiac α_2-adrenoceptors. Recently, B-HT 920 has been demonstrated to possess dopamine-receptor agonistic properties (Andén et al 1982). A presynaptic dopaminergic effect of B-HT 920 and *NN*-di-n-propyl-dopamine has been demonstrated in the vasculature of the rat by Wilffert et al (1984). It could therefore be suggested that B-HT 920 increases cardiac output in rats by increasing cardiac contractility to stimulation of dopamine receptors in the heart. However, pretreatment of the pithed rats with the dopamine receptor antagonist sulpiride (0·3 mg kg^{-1} i.v.) did not affect the increase in cardiac output to B-HT 920 (100 μg kg^{-1} i.v.). The dose of sulpiride used is sufficiently high to antagonize the presynaptic effect of *NN*-di-n-propyl-dopamine (Wilffert et al 1984). On the other hand, the increase in cardiac output by B-HT 920 in pithed rats was antagonized by yohimbine at a dose that selectively blocks α_2-adrenoceptors in rats (1 mg kg^{-1} i.v., Van Meel et al 1981). In view of these considerations, the increase in cardiac output induced by B-HT 920 in pithed rats must be attributed to venous constriction following activation of α_2-adrenoceptors.

Prazosin, at a dose that selectively blocks α_1-adrenoceptors in pithed rats (0·1 mg kg^{-1} i.v., Van Meel et al 1981), antagonized the increase in cardiac output induced by methoxamine. This result shows that the increase in cardiac output by methoxamine in pithed rats is the result of α_1-adrenoceptor stimulation. The contribution of a possible positive inotropism to the increase in cardiac output by methoxamine in pithed rats can as yet not be ruled out.

The mechanisms of vasoconstriction following α_2-adrenoceptor activation appeared to be different for arterial and venous vessels. Similar to the observations of Gerold & Haeusler (1983) with verapamil, the calcium antagonist nifedipine (1 mg kg^{-1}) did not diminish the increase in cardiac output after B-HT 920 (100 μg kg^{-1}) in our studies. Venous constriction after stimulation of α_2-adrenoceptors seems therefore, unlike α_2-mediated constriction of arterioles (for review see Van Meel et al 1982), independent of an influx of extracellular calcium.

In pithed cats, methoxamine, but not B-HT 920, increased cardiac output. In this animal model, a positive inotropic effect of methoxamine could be excluded, since dø/dt, a parameter positively correlated to cardiac contractility, did not change. Since the cardiac frequency did not change after methoxamine either, it is most likely that the increase in cardiac output in pithed cats induced by methoxamine is the result of venoconstriction initiated by α_1-adrenoceptor stimulation. The finding that B-HT 920 did not elicit an increase in cardiac output at all in pithed cats shows that in this species venoconstriction cannot be elicited upon stimulation of postjunctional α_2-adrenoceptors. Similar results have been obtained in ganglion-blocked dogs, in which methoxamine, but not the selective α_2-adrenoceptor agonist B-HT 933, causes an increase in cardiac output (Zandberg et al 1984).

It was found that nifedipine increased the cardiac output of pithed rats. This observation may be explained as follows. In pithed rats a high level of circulating angiotensin II (AII) is present (De Jonge et al 1982). Pressor responses to AII in this animal model are, like those to α_2-agonists, sensitive to calcium entry blockers (unpublished results). There are indications that AII predominantly constricts arterioles (Shepherd & Vanhoutte 1975). Nifedipine may thus dilate the AII-constricted arterioles of the pithed rat, which facilitates blood flow from the arterial to the venous bed and finally causes an increase of the preload of the heart.

In conclusion, the present study demonstrates that

obvious species differences exist with respect to the presence of functional α_2-adrenoceptors in the venous vascular bed.

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Effect of chloroquine and some other antimalarials on the immune mechanism in experimental animals

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The effects of chloroquine and some other antimalarials on the immune responses in experimental animals have been examined. Chloroquine and quinine caused significant decrease of serum anti-SRBC haemagglutination titre. Chloroquine lowered the serum IgM level and also reduced plaque-forming cells in the spleen of mice. The delayed-type hypersensitivity responses to SRBC and the passive cutaneous anaphylaxis were also diminished in rats treated with chloroquine. Thus, the immunosuppressant activity of chloroquine may explain its efficacy in various types of immune disorders.

It is well documented that prostaglandins are involved in various forms of experimental arthritis and rheumatoid arthritis and that chloroquine antagonizes these effects (Tietz & Chrisman 1975). Prostaglandins are also known to play a major part in the function of T- and B-lymphocytes (Pelus et al 1977) which are involved in the immune mechanism and inhibit lymphocyte transformation, possibly by stimulation of adenylate cyclase activity (Smith et al 1971). That the beneficial effect of antimalarials on various immune disorders could be due to their effects on the immune mechanism, remains to be explored. Hence, we have examined the effect of antimalarials on both humoral and cellular immune processes in experimental animals.

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Method

Groups of albino mice of either sex were immunized by intravenous injections of 1×10^9 sheep red blood cells (SRBC) on 0 day either alone or with different doses of chloroquine ($20\ mg\ kg^{-1}$), quinine ($20\ mg\ kg^{-1}$), mepacrine ($10\ mg\ kg^{-1}$) or primaquine ($15\ mg\ kg^{-1}$) given from 0–5th day of immunization; similar doses were also used by Ayitey Smith (1980). All mice were bled on the 6th day and the anti-SRBC haemagglutination titres were estimated (Ferrante et al 1979). Estimation of total serum protein (Varley et al 1980) was also made in each group to examine if the antimalarials interfered with protein synthesis. Estimation of serum haemagglutination titre in presence of 2-mercaptoethanol was made to determine the type of antibody synthesis affected by the drugs (Carpenter 1975). Determination of antibody forming cells in the spleen of mice immunized by SRBC (plaque forming cells) was by the method based on Jerne's plaque technique (1963) with modifications (Janah et al 1970).

Delayed-type hypersensitivity responses which are mediated through the T-lymphocytes were studied by the footpad thickness method (Liew 1977); here two groups of mice were primed with 1×10^8 SRBC subcutaneously (s.c.) in the back (day 0) and subsequently treated with either 0·9% NaCl (saline) or with chloroquine. Both these groups were challenged with SRBC (s.c.) in the hind footpad on day 5. The increase