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Differential inhibition of α_2-adrenoceptor-mediated pressor responses by (+)- and (−)-verapamil in pithed rats

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The inhibitory effects of (+)- and (−)-verapamil on the hypertensive responses brought about by the α_2-adrenoceptor agonist B-HT 920 were investigated in pithed normotensive rats. (−)-Verapamil was found to be about 4 times more potent with respect to depressing α_2-pressor responses compared with (+)-verapamil. To rule out the effect of 'unspecific' vasodilatation after the administration of the stereoisomers of verapamil, vasopressin was continuously infused into the carotid artery of pithed rats in a separate series of experiments. In the course of this vasopressin infusion, new inhibitory activities of the stereoisomers of verapamil on α_2-adrenoceptor-mediated pressor responses were determined. Under these circumstances, the potency ratio of (−)- vs (+)-verapamil was about 7. With the aid of a radioligand binding assay using [^{3}H]clonidine to identify α_2-adrenoceptors, low affinities were measured for the stereoisomers of verapamil. A K_i = 6170 nM for (−)-verapamil and a K_i = 41700 nM for (+)-verapamil were calculated. The results indicate that the interaction between α_2-adrenoceptor-mediated pressor responses and calcium entry blockers, such as verapamil, is a stereoselective event.

Both clinical and experimental evidence indicates that verapamil is a vasodilator which lowers total peripheral resistance and arterial blood pressure in addition to its myocardial depressant activity (for review, see Singh et al 1978). The basic mechanism involved in these effects of verapamil is presumed to be due to its selective inhibition of calcium influx into smooth muscle cells (Peiper et al 1971; Haeusler 1972; Singh et al 1978). The interaction between organic calcium entry blockers and calcium movements in vascular smooth muscle is very specific and there are indications that a certain degree of stereoselectivity is involved. Differential effects have been observed for the stereoisomers of D 600 (Jim et al 1981) and nimodipine (Towart et al 1982) on smooth muscle contraction after K^+-depolarization.

In previous reports we have indicated that calcium entry blockers selectively interfere with vasoconstrictor processes induced by postjunctional α_2-adrenoceptor stimulation (Van Meel et al 1981 a, b). Therefore, it seemed of interest to study the effects of (+)- and (−)-verapamil on the hypertensive responses brought about by the selective α_2-adrenoceptor agonist B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-[5,4-d]-azepin, Van Meel et al 1981c) in pithed rats. In addition, we have determined the affinity of the isomers of verapamil for α_2-adrenoceptors in binding experiments using [^{3}H]clonidine as a radioligand.

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MATERIALS AND METHODS

Male normotensive Wistar rats (200–250 g) were pithed under hexobarbitone-sodium anaesthesia (150 mg kg^{-1} i.p.) and subsequently ventilated via a tracheal cannula connected to a positive pressure pump. Rectal temperature was maintained at approximately 37 °C. Both carotid arteries were cannulated for the continuous registration of arterial pressure and for the administration of the calcium entry blockers. After the injection of the latter drugs, a 15-min period of stabilization was allowed.

Diastolic pressure was increased by administration of the α_2-adrenoceptor agonist B-HT 920 injected into the right jugular vein in a volume of 0·5 ml kg^{-1}. Log dose-pressor response curves were constructed by injecting the stimulant in a cumulative fashion. The determination of one complete dose-response curve lasted for approximately 5 min. During this period the depression of the response to a single dose of B-HT 920 was constant.

The inhibitory activities of the stereoisomers of verapamil as well as the racemic mixture on the α_2-adrenoceptor-mediated pressor responses were quantified in terms of −log ID50 values according to the method described by Van Rossum (1963).

Accordingly, the maximally attainable increases in diastolic pressure induced by B-HT 920 of the individual curves obtained in the presence of a stereoisomer of verapamil were plotted as percentage of the maximal vasopressor effect measured in the absence of the calcium entry blocker against the logarithm of the doses of the antagonist. Linear regression analysis was performed and the ID50-values of the stereoisomers calculated. ID50 is the dose (mol kg^{-1}) of the particular calcium antagonist producing a reduction of the maximal increase in diastolic pressure caused by B-HT 920 by 50% in pithed rats. The −log ID50-values are presented as mean values ±95% confidence intervals.

Binding studies were performed using [^{3}H]clonidine (26·7 Ci $mmol^{-1}$) as radioligand. Brain membrane preparations of normotensive Wistar rats (200–250 g) were prepared as reported elsewhere (Timmermans et al 1981). The specific binding of [^{3}H]clonidine was defined as the excess over blanks containing 10 μM of (−)-noradrenaline. The inhibition of the specific binding of [^{3}H]clonidine (0·4 nM) was examined in the presence of various concentrations of unlabelled clonidine as well as the stereoisomers of verapamil. Incubations were performed in Tris/HCl buffer (pH = 7·7) at 25 °C for 60 min in a final volume of 1 ml. Final protein concentration amounted to 2 mg ml^{-1} for the [^{3}H]clonidine assays. Rapid vacuum filtration through Whatman GF/B filters terminated the incubations. Filters were washed by three 5-ml portions of ice-cold Tris/HCl buffer and counted for radioactivity. The concentration reducing the specific binding of [^{3}H]clonidine by 50% (IC50) was calculated from the equation: K_i = IC50/(1 + [radioligand]/K_D), where K_D = 3·6 nM (Timmermans et al 1981).

Drugs used were: B-HT 920. 2HCl (Thomae); clonidine HCl and [^{3}H]clonidine HCl (26·7 Ci $mmol^{-1}$) (Boehringer Ingelheim); (−)-noradrenaline (Sigma); (±)-verapamil HCl, (+)-verapamil HCl and (−)-verapamil HCl (Knoll).

All substances were dissolved in 0·9% NaCl (saline). The doses mentioned in the text refer to the forms indicated above.

Statistics: results are given as mean values ±s.e.m. Analysis of variance and Student's *t*-test for unpaired observations were used to ascertain statistical significance ($P < 0·05$).

RESULTS

In pithed normotensive Wistar rats, B-HT 920 (1–1000 μg kg^{-1}) produced a maximal increase in diastolic pressure of 94·5 ± 2·2 mm Hg (n = 10). The maximal plateau of the increase in diastolic pressure induced by the activation of α_2-adrenoceptors was progressively reduced by (±)-verapamil (also see Van Meel et al 1981b) and its stereoisomers. A non-competitive type of interaction was observed for these calcium entry blockers (see Figs 1, 2, 3).

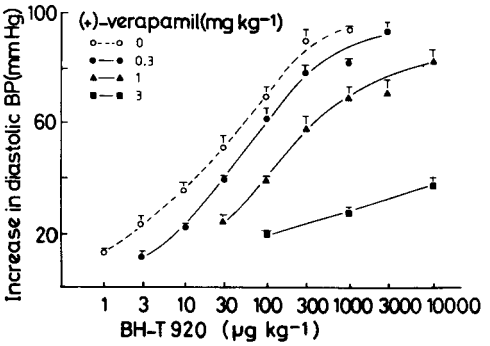

FIG. 1. Log dose-vasopressor response curves of B-HT 920 with respect to the increase in diastolic pressure in the absence and the presence of (+)-verapamil in pithed, normotensive rats. Symbols represent mean values ± s.e.m. (n = 5–7). Initial mean value of diastolic pressure was 39·1 ± 1·0 mm Hg (n = 26).

After calculation of the −log ID50-values of the verapamil isomers, the following sequence of potency with respect to the depression of α_2-adrenoceptor-induced pressor effects was observed: (−)-verapamil (−log ID50 = 5·87 ± 0·19) ≥ (±)-verapamil (−log ID50 = 5·78 ± 0·12) > (+)-verapamil (−log ID50 = 5·27 ± 0·09). (−)-Verapamil was significantly ($P < 0·05$) more potent than (+)-verapamil; the potency ratio was about 4.

Vasodilatation as such has been noted to attenuate α_2-adrenoceptor-mediated pressor responses to some extent (De Jonge et al 1982). In the model of

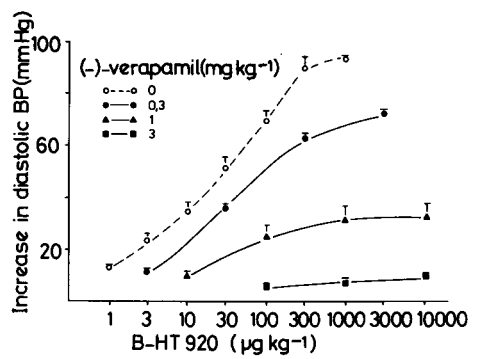

FIG. 2. Log dose-vasopressor response curves of B-HT 920 with respect to the increase in diastolic pressure in the absence and the presence of (−)-verapamil in pithed, normotensive rats. Also see the legend to Fig. 1.

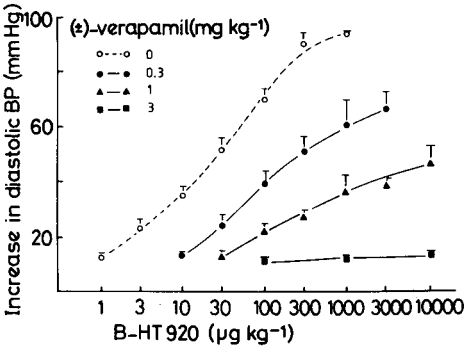

FIG. 3. Log dose-vasopressor response curves of B-HT 920 with respect to the increase in diastolic pressure in the absence and the presence of (±)-verapamil in pithed, normotensive rats. Also see the legend to Fig. 1.

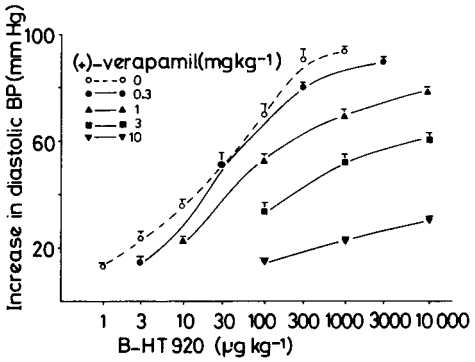

FIG. 4. Log dose-vasopressor response curves of B-HT 920 with respect to the increase in diastolic pressure in the absence and presence of (+)-verapamil in the course of a vasopressin infusion in pithed normotensive rats. Symbols represent mean values ± s.e.m. (n = 5–7). The mean value of the diastolic pressure after stabilization of the vasopressin effect amounted to 62 ± 2 mm Hg (n = 32).

the pithed rat, verapamil and its isomers decreased diastolic pressure. Therefore, we also examined the interaction between (+)- and (−)-verapamil and α_2-adrenoceptor-mediated pressor responses in the course of a vasopressin infusion. In all experiments, the diastolic pressure of the pithed rats after administration of the slow calcium channel blockers was gradually increased to a level of 60–65 mm Hg by adjusting the rate of infusion of vasopressin (0·1 iu ml^{-1}). A rise in diastolic pressure by vasopressin to a level of 60–65 mm Hg as such had no significant effect on the vasoconstrictor responses to B-HT 920 (results not shown). Under these circumstances a new −log ID50 = 5·85 ± 0·21 and a significantly ($P < 0·05$) smaller new value of 5·02 ± 0·11 were calculated for (−)- and (+)-verapamil, respectively. The potency ratio of (−)- over (+)-verapamil was now about 7. The effect of vasopressin infusion on the inhibitory activities of (+)- and (−)-verapamil on α_2-adrenoceptor-mediated pressor responses is shown in the Figs 4 and 5.

Radioligand binding experiments were performed to determine the affinities of the stereoisomers of verapamil for α_2-adrenergic binding sites labelled with [^{3}H]clonidine. The potencies of (−)-, (+)- and (±)-verapamil in displacing [^{3}H]clonidine from its specific binding sites in rat brain were rather weak. The following K_i-values (nM) were calculated: (+)-verapamil 41700, (−)-verapamil 6170 and (±)-verapamil 22 100 (also see Van Meel et al 1981b).

DISCUSSION

Differential effects on contraction of smooth muscle have been reported for stereoisomers of calcium entry blockers. In-vitro, a marked difference in activity was observed for (+)- and (−)-nimodipine (Towart et al 1982) and (+)- and (−)-methoxyverapamil (Jim et al 1981) on smooth muscle contraction. It was consistently found that the (−)-isomers were more potent than the (+)-isomers (10–40 times) with respect to the inhibition of smooth muscle contraction after K^+-depolarization. These results suggest that the inhibition of the transmembrane influx of extracellular calcium into smooth muscle cells after K^+-depolarization is a stereoselective event.

In the cardiovascular system of pithed rats, vascular postjunctional α_1- and α_2-adrenoceptors both induce vasoconstriction upon stimulation (for review see Timmermans & Van Zwieten 1981). The α_2-adrenoceptor-mediated vasopressor effects can be depressed selectively by calcium entry blockers,

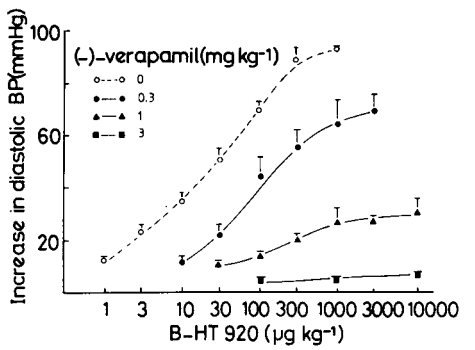

FIG. 5. Log dose-vasopressor response curves of B-HT 920 with respect to the increase in diastolic pressure in the absence and presence of (−)-verapamil in the course of a vasopressin infusion in pithed normotensive rats. Symbols represent mean values ± s.e.m. (n = 5–7). Also see the legend to Fig. 4.

whereas the hypertensive effects induced by α_1-adrenoceptor activation are hardly affected (Van Meel et al 1981a, b). (±)-Verapamil (Van Meel et al 1981b) and its isomers (this study) depressed the α_2-adrenoceptor-mediated pressor responses. It was found that the (−)-isomer of verapamil was significantly more potent than the (+)-isomer. Similar differential effects of the stereoisomers of verapamil have been observed with respect to coronary vasodilatation. When administered intraveneously to anaesthetized open-chest dogs, (−)-verapamil was about twice as potent as (+)-verapamil in increasing coronary sinus flow and in decreasing mean arterial pressure and coronary resistance (Satoh et al 1980). This suggests that (−)-verapamil possesses a more pronounced vasodilator activity than the (+)-isomer. These findings are in accordance with the effects of (−)- and (+)-verapamil observed on α_2-pressor responses in-vivo (present study) and would be in agreement with the view that vasodilatation and depression of α_2-pressor responses are related to each other.

The radioligand displacement studies showed that the stereoisomers of verapamil exerted different affinities for α_2-adrenoceptor binding sites. The (−)-isomer proved to be somewhat more potent than the (+)-isomer of verapamil in displacing [^{3}H]clonidine from its specific binding sites. However, the affinities of the stereoisomers of verapamil are very low and therefore cannot explain the vasodilator properties of these compounds. Recently, tritiated calcium entry blockers such as [^{3}H]nitrendipine (Bellemann et al 1981; Murphy & Snyder 1982) and [^{3}H]nimodipine (Bellemann et al 1982) have been used to demonstrate specific binding sites for calcium entry blockers in the heart, brain and other tissues. It appeared that the (−)-enantiomers of calcium entry blockers were always more potent in displacing [^{3}H]nitrendipine or [^{3}H]nimodipine in comparison with the (+)-isomers (Bellemann et al 1982). In addition, it was found that the rank order of potency for displacing [^{3}H]nitrendipine from its specific binding sites for structurally different calcium entry blockers was: felodipine = nimodipine = nisoldipine > nifedipine $\gg$ D 600 > diltiazem > verapamil (Murphy & Snyder 1982). Obviously, the order of potency of these substances assayed in these binding experiments does not fully correspond with the pharmacological data, such as their inhibitory activity on K^+-induced contractions (Van Meel et al 1983). Evaluation of the displacement experiments of [^{3}H]nimodipine by different 1,4-dihydropyridines, yielded the sequence: nimodipine $\geqslant$ nisoldipine = nifedipine > nitrendipine (Bellemann et al 1982) which does not correspond with the activities observed in pharmacological studies such as the inhibition of K^+-induced contractions or depression of α_2-pressor responses (Van Meel et al 1983). It seems very attractive to use tritiated 1,4-dihydropyridines as selective markers for 'calcium channels', but it must be concluded that these radioligand binding experiments cannot directly be translated into the known pharmacological actions of the calcium entry blockers yet. One of the possible reasons may be that the various calcium channels in the different tissues are not identical. In this respect it is well established that the calcium channels in the heart and the blood vessels differ (Fleckenstein & Roskamm 1980).

This and other studies have observed a stereoselective interaction between the various calcium entry blockers (verapamil, D 600, nimodipine) and receptor-operated and membrane-potential-operated calcium channels, respectively. These findings suggest that separation of the stereoisomers of calcium entry blockers may yield more potent calcium antagonistic compounds. Obviously, the difference in potency (expressed as -log ID50 values) between (+)- and (−)-verapamil is significant, but small. However, resolution of the optical isomers of, for example, nimodipine (Towart et al 1982) may improve the calcium antagonistic properties to a greater extent and consequently the vasodilator activity. It would therefore seem of interest to separate, where appropriate, the stereoisomers of calcium entry blockers in order to obtain more active and specifically acting vasodilators.

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