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Inhibition of Uptake$_1$ by dopexamine hydrochloride *in vitro)*

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1 Dopexamine hydrochloride, a compound under evaluation for the acute treatment of heart failure, was examined *in vitro* for its ability to prevent neuronal uptake of noradrenaline.

2 Despite possessing only weak β_1-adrenoceptor agonist activity in paced guinea-pig left atria, dopexamine hydrochloride was only 23 times less potent than isoprenaline in augmenting responses of field-stimulated atrial preparations.

3 This potent effect was not observed in field-stimulated atria depleted of noradrenaline by reserpine and in the presence of cocaine was greatly reduced (1 μM) or abolished (50 μM).

4 Dopexamine hydrochloride (3 μM) potentiated the inotropic effect of exogenous noradrenaline in paced atria, thereby resembling cocaine (10 μM) and dopamine (30 μM), both of which are known inhibitors of Uptake$_1$.

5 The sodium-dependent uptake of [^{3}H]-noradrenaline into rabbit brain synaptosomes was prevented by dopexamine hydrochloride (IC_{50} 26 nM) and cocaine (IC_{50} 108 nM), as well as by two other catecholamines used in the treatment of heart failure, dopamine (IC_{50} 270 nM) and dobutamine (IC_{50} 380 nM).

6 The cardiac stimulant effect of dopexamine hydrochloride reported in dogs and in patients with heart failure, may therefore be due in part to potentiation of endogenous catecholamines.

Introduction

Dopexamine hydrochloride is an agonist at peripheral dopamine receptors and β_2-adrenoceptors but possesses only weak β_1-adrenoceptor agonist activity (Brown *et al.*, 1985a). This novel pharmacological profile results in cardiovascular effects, namely afterload reduction, improvement of renal perfusion and cardiac stimulation, which are likely to be useful in the treatment of low cardiac output states (Brown *et al.*, 1985b).

In contrast to the weak β_1-adrenoceptor agonist activity demonstrated in spontaneously beating atria with a relative intrinsic activity one tenth that of isoprenaline (Brown *et al.*, 1985a), dopexamine hydrochloride was found to be a potent stimulant in preliminary experiments conducted in field-stimulated left atria. This unexpected result prompted the present investigation.

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Methods

Guinea-pig atria

Guinea-pig left atria was suspended in 30 ml baths containing Krebs-Henseleit solution of the following composition (mM): NaCl 117.6, KCl 5.4, $NaHCO_3$ 25.0, $CaCl_2$ 2.55, $MgSO_4$ 1.12, NaH_2PO_4 0.9, D-glucose 11.1 and L-ascorbic acid 1.1, kept at 37°C and gassed with 95% O_2 5% CO_2. Each atria was tied to a tissue-hook electrode at the end of a tissue holder, and the other end was attached to an overhead force transducer (Grass, FT03C). The tissues were set initially to a diastolic tension of 2 g and paced (Grass S88 Stimulator) with pulses of 1 Hz, 5 ms duration and 1.5 times the threshold voltage (typically 5–10 V). The stimulating voltage was raised to an arbitrary value of 50 V in some experiments to elicit simultaneous pacing and field-stimulation of the atria.

The atria were initially exposed to cumulatively added concentrations of isoprenaline (0.3 nM – 1 μM) and the maximal increase in tension above baseline

expressed as 100%. All subsequent inotropic responses, either induced by increasing the stimulating voltage to give field-stimulation, or by addition of drug, are expressed as a percentage of the maximal isoprenaline-induced response.

Dopexamine

The tension changes induced in the presence of dopexamine hydrochloride (10 nM–100 μM) on atria either paced at 50% above the threshold voltage or field-stimulated, are expressed as a percentage of the maximum isoprenaline-induced rise in tension. The response to dopexamine hydrochloride was re-examined in field-stimulation experiments 15 min after either the addition of the selective β_2-adrenoceptor antagonist ICI 118551 (30 nM, $n = 3$; Bilski *et al.*, 1983) or in the presence of the non-selective β-adrenoceptor antagonist propranolol (1 μM, $n = 4$). Field-stimulation experiments were also performed on atria taken from guinea-pigs depleted of noradrenaline (5 mg kg^{-1} reserpine, i.p. 24 h previously, Crout *et al.*, 1962).

Cocaine

The inotropic effect of the $Uptake_1$ blocker, cocaine, was investigated by cumulative addition (1–50 μM) to paced and field-stimulated atria. In addition, the effect of either a single administration of 1 μM or of cumulatively added 50 μM cocaine (30 min incubation) on the potent inotropic effect of dopexamine hydrochloride was examined in field-stimulated atria.

In another series of experiments, the effect of cumulatively added cocaine was examined in field-stimulated atria in the presence of 3 μM dopexamine hydrochloride (30 min incubation).

Response to exogenous noradrenaline

The influence of dopexamine hydrochloride on the cumulative inotropic response curve to noradrenaline (1 nM–100 μM) was investigated on paced atria. Following recovery from the control responses to noradrenaline, dopexamine hydrochloride (3 μM) was added and 30 min later a noradrenaline cumulative response curve was re-established. Values are expressed as a percentage of the control maximum increase in tension produced by noradrenaline. In other experiments dopexamine hydrochloride was substituted by $Uptake_1$ blocking concentrations of either cocaine (10 μM) or dopamine (30 μM). In view of the inotropic response produced by each of these agents on its own, noradrenaline (3 μM) was substituted for the drugs described above to simulate the rise in basal tension prior to the cumulative addition of noradrenaline. The reproducibility of the noradrenaline response was also checked by substitution of saline for the test compound.

Preparation of synaptosomes

Synaptosomes were prepared from rabbit brain cerebral cortex by the method of Gray & Whittaker (1962). Tissue was homogenized in ice-cold 0.32 M sucrose with a glass/teflon homogenizer and centrifuged to remove cell debris (1,000 g_{av}, 10 min, 4°C). The supernatant was centrifuged at 12,000 g_{av} for 20 min at 4°C and the pellet, P2, (Gray & Whittaker, 1962) resuspended in 0.32 M sucrose. For some experiments this crude synaptosomal fraction was used. In others, the P2 suspension was fractionated on discontinuous sucrose gradients in a swing-out rotor (100,000 g_{av} for 1 h at 4°C). Material at the 0.8/1.2 M sucrose interface (P2b) was collected and diluted with 0.32 M sucrose to a 2 ml g^{-1} wet weight of original tissue. Synaptosomal suspensions were kept on ice and used within 2 h of preparation.

Inhibition of [^{3}H]-noradrenaline uptake

Incubations were carried out in Tris-buffered Krebs solution (Wood & Wyllie, 1983) of the following composition (mM): NaCl 136.0, KCl 5.0, $MgCl_2$ 1.2, $CaCl_2$ 2.5, D-glucose 10.0, L-ascorbate 1.0, Tris 20.0, adjusted to pH 7.4 with HCl (at 37°C). To allow correction for noradrenaline uptake not due to $Uptake_1$, a buffer in which NaCl was replaced by equimolar LiCl was employed. Compounds were preincubated with synaptosomes (0.1 ml) for 5 min at 37°C in a volume of 0.9 ml. The $Uptake_1$ process was initiated by the addition of 0.1 ml [^{3}H]-noradrenaline to give a final concentration of 50 nM, 0.1 μCi ml^{-1}. After a further 5 min incubation, the process was terminated by the addition of 2 ml buffer at room temperature followed by immediate filtration through Whatman GF/B filters under reduced pressure and washing with a further 2 ml buffer. Filters were soaked overnight in optiphase scintillation cocktail (Fisons) before counting. Uptake was never more than 5% of the total [^{3}H]-noradrenaline added to the fractions under these conditions.

Drugs

The following drugs were used: dopexamine hydrochloride (Dopacard, Fisons plc), cocaine hydrochloride (May & Baker), dobutamine hydrochloride (Lilly), ICI 118551 (ICI, erythro-(±)-1-(7-methylindom-4-yloxy)-3-isopropyl-aminobutan-2-ol, isoprenaline hydrochloride (Pharmax), dopamine hydrochloride and noradrenaline base (Sigma), noradrenaline acid tartrate (Winthrop), reserpine (Aldrich) and (−)-[7,8-^{3}H]-noradrenaline, 15 Ci $mmol^{-1}$ (Amer-

sham). All of the compounds were dissolved in saline, ascorbic acid (0.02% w/v) acting as the anti-oxidant. Reserpine was dissolved in 20% (w/v) ascorbic acid to give a concentration of 10 mg ml^{-1} for injection (Crout *et al.*, 1962).

Results are calculated as the mean ± s.e.mean and the unpaired or paired Student's *t* test used (1 tailed) to determine significance.

Results

Field-stimulated atria

Raising the pacing voltage to 50 V from 6–9 V (50% above the threshold voltage), increased ($P < 0.05$) the 'all or none' developed tension from 0.71 ± 0.22 g ($n = 7$) to 1.27 ± 0.33 g; 42 ± 10% of the maximum increase in tension (1.51 ± 0.20 g) produced by isoprenaline. This increase was absent in reserpinised atria (3.5 ± 3.7%, NS) despite a normal isoprenaline response (maximum tension rise of 2.10 ± 0.32 g, $n = 4$). The effectiveness of reserpinisation was demonstrated by the loss of the tyramine (80 μM) response during low-voltage stimulation which was 91.3 ± 13.6% ($P < 0.01$) in normal and 12.8 ± 6.3% (NS) in reserpinised atria of the maximal rise in tension induced by isoprenaline.

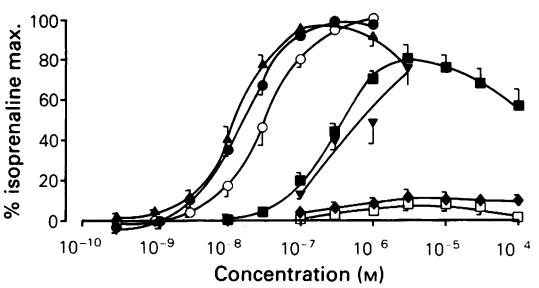

Figure 1 Dopexamine hydrochloride was a potent stimulant in field-stimulated guinea-pig atria (■, $n = 10$) in contrast to its relatively weak action in atria stimulated at 1.5 times the threshold voltage (□, $n = 8$). No such distinction was evident with isoprenaline in either type of atrial preparation (●, $n = 10$ and ○, $n = 8$ respectively). The potent effect of dopexamine hydrochloride was not affected when repeated in the presence of the β_2-adrenoceptor antagonist ICI 118551 at a concentration of 30 nM (▼, $n = 3$) but it was abolished by 1 μM propranolol ($n = 4$, not shown). The dependence of the potent response of dopexamine hydrochloride upon endogenous noradrenaline was shown by the loss of activity in reserpinised field-stimulated atria (◆, $n = 4$), whilst the isoprenaline response (▲, $n = 4$) was unaffected. Values are means with s.e.mean shown by vertical lines.

Dopexamine hydrochloride

In atria placed at 1.5 times the threshold voltage (9 ± 1 V, $n = 8$), the basal developed tension was 0.32 ± 0.07 g and the maximum increase in tension induced by isoprenaline above basal tension was 1.40 ± 0.20 g. In these atria, dopexamine hydrochloride produced only a small rise in developed tension of up to 8% of the maximum isoprenaline response (Figure 1).

In field-stimulated atria ($n = 10$), the basal developed tension was significantly higher (1.55 ± 0.20 g), but the maximal increase in tension produced by isoprenaline was similar (1.58 ± 0.14 g) to the results obtained in paced atria. In contrast, dopexamine hydrochloride markedly increased tension, reaching 81 ± 6% of the isoprenaline-induced maximum tension rise at 3 μM, approximately 23 times weaker than isoprenaline (Figure 1). This action was unaffected by the presence of ICI 118551 (30 nM; $n = 3$; Figure 1) but abolished by propranolol (1 μM; $n = 4$; not shown). In field-stimulated atria taken from reserpinised guinea-pigs, the basal tension (0.50 ± 0.11 g, $n = 4$) was smaller and dopexamine hydrochloride gave only a weak response, characteristic of paced atria, despite a normal isoprenaline response (maximum rise in tension of 2.10 ± 0.32 g, Figure 1).

Cocaine

Cumulatively added cocaine was inactive in paced atria ($n = 4$) but produced a bell-shaped inotropic response curve in field-stimulated atria, the maximum response being approximately half that elicited by isoprenaline (Table 1). The highest concentration (50 μM) of cocaine used did not impair atrial contractility as assessed by the response to isoprenaline.

Cocaine-dopexamine interactions

In field-stimulated atria, dopexamine hydrochloride (0.3 μM) which produced an initial rise in tension of 37 ± 4% of the maximal increase induced by isopren-

Table 1 The inotropic effect of cocaine in field-stimulated guinea-pig atria, in the absence or presence of 0.3 μM dopexamine hydrochloride

Treatment	*Concentration (μM)*					
	(*n*)	1	5	10	20	50
Control	(7)	17 ± 4	39 ± 5	45 ± 6	42 ± 5	25 ± 5
Dopexamine	(5)	10 ± 4	23 ± 4*	25 ± 5*	12 ± 5**	3 ± 5**

Values are mean ± s.e.mean of isoprenaline-induced maximum.
Student's *t* test; *$P < 0.05$; **$P < 0.01$.

aline, declining to 20 ± 6% ($n = 5$) 30 min later, virtually halved the response to cumulatively added cocaine (Table 1). Conversely the inotropic response to cumulatively added dopexamine hydrochloride was significantly depressed in field-stimulated atria by cocaine. In the presence of 50 μM cocaine ($n = 7$) the response was abolished whilst in atria exposed to 1 μM cocaine, the maximum rise in tension to dopexamine hydrochloride (3 μM) was 27 ± 10% of the isoprenaline-induced maximum ($n = 5$), significantly lower than in the absence of cocaine (81 ± 6%, $P < 0.01$, $n = 10$) and significantly greater than in paced atria (8 ± 3%, $P < 0.05$, $n = 8$).

Exogenous noradrenaline response

In paced atria, noradrenaline (1 nM–100 μM) produced a rise in tension. Following recovery, 30 min preincubation with either dopexamine hydrochloride (3 μM), cocaine (10 μM) or dopamine (30 μM), caused an increase in basal tension amounting to 21 ± 11%, 27 ± 10% or 15 ± 14% respectively of the preceding (control) maximum response to noradrenaline. In each case the response curve to cumulatively added noradrenaline was shifted left-ward, 25 fold in the case of dopexamine hydrochloride ($P < 0.05$), with no significant change in the maximum response to noradrenaline (Figure 2), contrasting with a 2 fold rightward shift ($P < 0.05$) in the absence of drug (Figure 3). In the presence of noradrenaline (3 μM) to produce a rise in basal tension (28 ± 5% of the preceding noradrenaline maximum response) the response to cumulatively added noradrenaline was not potentiated but was additive, showing convergence at concentrations greater than 3 μM (Figure 3).

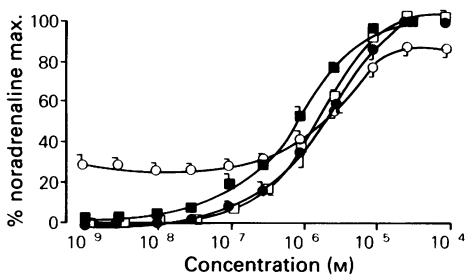

Figure 3 Guinea-pig atria paced at 1.5 times the threshold voltage gave a reproducible response to cumulatively added noradrenaline in the presence of saline (□) and only an additive effect was produced in the presence of 3 μM noradrenaline (○) given 30 min previously. Control responses are the corresponding filled symbols. Values are means with s.e.mean shown by vertical lines $n = 4$.

Inhibition of [^{3}H]-noradrenaline uptake

Dopexamine hydrochloride produced a potent, concentration-dependent inhibition of the sodium-dependent [^{3}H]-noradrenaline uptake by rabbit brain synaptosomes (Figure 4). The IC_{50} was 26 nM (geometric mean of 5 independent experiments with 95% confidence limits of 9–75 nM). Dopexamine hydrochloride was found to be more potent than cocaine, IC_{50} 108 nM ($n = 5$, 64–180 nM), dopamine IC_{50} 270 nM ($n = 4$, 208–342 nM) and dobutamine IC_{50} 380 nM ($n = 4$, 110–1290 nM). By contrast, isoprenaline was only a weak inhibitor, with an IC_{50} of 9.8 μM ($n = 4$, 6.1–16 μM). Additional experiments with non-purified synaptosomes gave similar results and combining the data produced an IC_{50} for dopex-

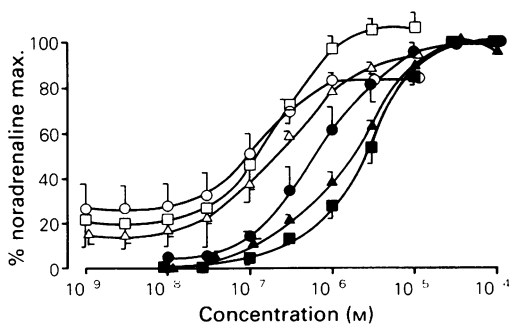

Figure 2 In guinea-pig atria paced at 1.5 times the threshold voltage, the stimulant effect of cumulatively added noradrenaline was potentiated 30 min after the addition of either 3 μM dopexamine hydrochloride (□), 10 μM cocaine (○) or 30 μM dopamine (△). Control noradrenaline responses are shown by the corresponding filled symbols. Values are means with s.e.mean shown by vertical lines $n = 4$.

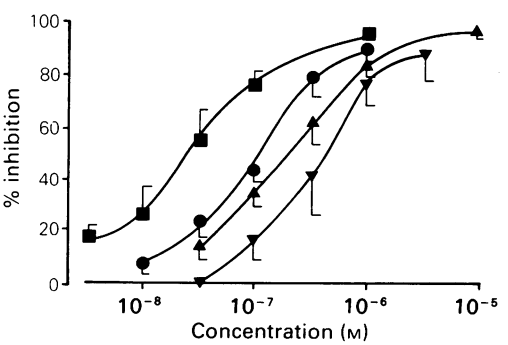

Figure 4 Inhibition of [^{3}H]-noradrenaline uptake into purified rabbit brain synaptosomes by dopexamine hydrochloride (■), cocaine (●), dopamine (▲) and dobutamine (▼). Values are the mean with s.e.mean (shown by vertical lines) of 4 or 5 experiments. The control sodium-dependent noradrenaline uptake was 497 ± 169 fmol min^{-1}mg^{-1} protein.

amine hydrochloride of 18.3 nM ($n = 10$, 10.5–32 nM), cocaine IC_{50} 106 nM ($n = 10$, 85–132 nM), dopamine IC_{50} 258 nM ($n = 8$, 198–336 nM), dobutamine IC_{50} 364 nM ($n = 8$, 221–597 nM) and isoprenaline IC_{50} 10.1 μM ($n = 10$, 8.4–12.1 μM).

Discussion

The weak β_1-adrenoceptor agonist activity of dopexamine hydrochloride reported previously in spontaneously beating guinea-pig atria (Brown *et al.*, 1985a) was confirmed in the present study in paced left atria. In contrast, under conditions of field-stimulation, dopexamine hydrochloride was a potent stimulant. The inability of ICI 118551 to prevent this potent response excluded either the involvement of postjunctional inotropic or prejunctional facilitatory β_2-adrenoceptor stimulation. However, the effect was mediated by β_1-adrenoceptor stimulation since it was completely abolished by the non-selective β-adrenoceptor antagonist propranolol. In addition the response was dependent upon the presence of endogenous noradrenaline since it was absent in reserpinised field-stimulated atria.

These results strongly suggest an indirect sympathomimetic effect mediated by endogenous noradrenaline. Inhibition of Uptake$_1$ was suspected, based on the similarity to cocaine, a known blocker of the Uptake$_1$ process (Iversen, 1967) which was inotropically inactive in paced atria, but produced a rise in tension in field-stimulated atria, confirming observations made by Blinks (1966). However, the effect of cocaine was optimal at 10 μM; above this concentration, tension fell. A fall in noradrenaline output at these high concentrations may result from a local anaesthetic effect (Hughes, 1972), and this may explain why dopexamine hydrochloride produced a disproportionately greater inotropic response than cocaine relative to Uptake$_1$ inhibition. For example, at 1 μM dopexamine hydrochloride or cocaine produced inotropic responses of 70% and 17%, and inhibition of Uptake$_1$ of 96% and 91% respectively. In addition the direct, though weak, β_1-adrenoceptor stimulant property of dopexamine hydrochloride would be expected to exacerbate this difference. In support of this hypothesis is the fact that dopexamine hydrochloride and cocaine both produce their maximal effects on inotropy and Uptake$_1$ at similar respective concentrations (3 and 10 μM).

Cocaine attenuated and abolished the inotropic response to dopexamine hydrochloride at 1 and 50 μM respectively without directly impairing atrial contractility. These concentrations would be expected to inhibit Uptake$_1$ by 90 and 100% respectively. The most likely conclusion is that prior blockade of Uptake$_1$ with cocaine prevented the effect of dopexamine hydrochloride. Similarly dopexamine hydrochloride at a concentration of 0.3 μM, which produced about 87% inhibition of Uptake$_1$ in rabbit brain synaptosomes, halved the inotropic response to cocaine. A higher concentration of dopexamine hydrochloride was not used since the powerful inotropic effect which it would elicit would make difficult the interpretation of the response to cocaine.

Due to their potent direct sympathomimetic properties at the β_1-adrenoceptor, neither dopamine (Brown *et al.*, 1985a) nor dobutamine (Offermier *et al.*, 1976) could be contrasted in paced and field-stimulated atria. However dopamine, and concentrations of dopexamine hydrochloride or cocaine which were active in the field-stimulated atria, potentiated the inotropic effect of exogenous noradrenaline in paced atria. The concentrations used were approximately 100 times their respective IC_{50} concentrations at inhibiting [^{3}H]-noradrenaline uptake into rabbit brain synaptosomes and would be expected to produce maximal inhibition of Uptake$_1$. Each of these agents alone also caused a small rise in developed tension. Since cocaine is not a directly acting sympathomimetic, a small degree of field-stimulation was probably present in these paced atrial experiments. However, this small rise in developed tension *per se* was not responsible for the potentiation of the noradrenaline response since prior addition of noradrenaline to mimic the tension rise, resulted only in an additive effect.

Confirmation that dopexamine hydrochloride prevents neuronal uptake of noradrenaline was demonstrated by the potent inhibitory effect on [^{3}H]-noradrenaline sequestration into rabbit brain synaptosomes. This uptake process had the characteristics of Uptake$_1$ in being dependent upon the presence of sodium ions, inhibited by cocaine and poorly inhibited by isoprenaline as previously reported by Iversen (1967). The cardiac stimulant effect of dopexamine hydrochloride observed in the dog was previously reported to be a consequence of β-adrenoceptor stimulation since it was prevented by propranolol (Brown *et al.*, 1985b). We have also shown that the cardiac stimulant effect of dopexamine hydrochloride in the dog, in addition to baroreflex activation, is also due to cardiac β_2-adrenoceptor stimulation, blocked by ICI 118551 (Smith *et al.*, 1987). However, in view of the present findings, potentiation of endogenous catecholamines, as has been demonstrated for cocaine in the anaesthetized dog (Brown *et al.*, 1979), must be considered as an additional mechanism for the inotropic response of dopexamine.

In support of this possibility, Bass *et al.* (1986) recently reported that intravenous infusion of dopexamine hydrochloride in the anaesthetized dog, attenuated the cardiovascular responses to tyramine, whilst potentiating those to noradrenaline. These findings

concur with results of experiments conducted by ourselves in both the anaesthetized cat and dog, in which the cardiac effects of noradrenaline and cardiac sympathetic nerve stimulation were potentiated by dopexamine hydrochloride with an attenuation of tyramine, thereby resembling the effect of cocaine and desmethylimipramine (unpublished observations). In none of our animal studies with dopexamine hydrochloride has vasoconstriction occurred, a possible vascular consequence of Uptake$_1$ blockade. It is likely that vasodilatation prevails due to stimulation of the vascular β_2-adrenoceptors and DA$_1$-receptors.

An afterload-reducing agent and renal vasodilator with positive inotropic activity, dopexamine hydrochloride improves cardiac function in patients with heart failure (Dawson *et al.*, 1985; Svensson *et al.*, 1986) and suspected coronary artery disease (Jaski *et al.*, 1986). Plasma levels typically obtained in man (0.02–0.6 μM; internal reports and Jaski *et al.*, 1986) and in anaesthetized dogs (0.08–0.4 μM; Dr D Hall, personal communication) with therapeutic-dose infusions of dopexamine hydrochloride, would on the basis of these *in vitro* studies, be within the concentration range able to produce Uptake$_1$ block, and it is suggested that the inotropic response is partially mediated by Uptake$_1$ blockade and hence potentiation of endogenous noradrenaline. The ability to block neuronal uptake of noradrenaline is shared by two other clinically used positive inotropic catecholamines, dopamine (Iversen, 1971) and dobutamine, although these are at least ten fold weaker than dopexamine hydrochloride. Enhancement of the activity of endogenous sympathetic mechanisms in the heart may be a useful alternative to continuous β_1-adrenoceptor stimulation in the treatment of heart failure.

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References

BASS, A.S., KOHLI, J.D., LUBBERS, N.L. & GOLDBERG, L.I. (1986). Cardiovascular evaluation of dopexamine, an unusual dopamine receptor agonist. *Clin. Res.*, **34**, 941A.

BILSKI, A.J., HALLIDAY, S.E., FITZGERALD, J.D. & WALE, J.L. (1983). The pharmacology of a β_2-selective adrenoceptor antagonist (ICI 118551). *J. cardiovasc. Pharmac.*, **5**, 430–437.

BLINKS, J.R. (1966). Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. *J. Pharmac. exp. Ther.*, **151**, 221–235.

BROWN, R.A., SOLCA, A.M. & WILSON, J. (1979). Catecholamine uptake blockade in anaesthetized dogs: influence on cardiovascular responses. *J. Pharm. Pharmac.*, **31**, 762–766.

BROWN, R.A., DIXON, J., FARMER, J.B., HALL, J.C., HUMPHRIES, R.G., INCE, F., O'CONNOR, S.E., SIMPSON, W.T. & SMITH, G.W. (1985a). Dopexamine: a novel agonist at peripheral dopamine receptors and β_2-adrenoceptors. *Br. J. Pharmac.*, **85**, 599–608.

BROWN, R.A., FARMER, J.B., HALL, J.C., HUMPHRIES, R.G., O'CONNOR, S.E. & SMITH, G.W. (1985b). The effects of dopexamine on the cardiovascular system of the dog. *Br. J. Pharmac.*, **85**, 609–619.

CROUT, J.R., MUSKUS, A.J. & TRENDELENBURG, U. (1962). Effect of tyramine on isolated guinea-pig atria in relation to their noradrenaline stores. *Br. J. Pharmac.*, **18**, 600–611.

DAWSON, J.R., THOMPSON, D.A., SIGNY, M., JUUL, S.M., TURNBULL, P., JENKINS, B.S. & WEBB-PEPLOE, M.M. (1985). Acute haemodynamic and metabolic effects of dopexamine: a new dopaminergic receptor agonist in patients with chronic heart failure. *Br. Heart. J.*, **54**, 313–320.

GRAY, E.G. & WHITTAKER, V.P. (1962). The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat.*, **96**, 79–88.

HUGHES, J. (1972). Evaluation of mechanisms controlling the release and activation of the adrenergic transmitter in the rabbit portal vein and vas deferens. *Br. J. Pharmac.*, **44**, 472–491.

IVERSEN, L.L. (1967). *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*. Cambridge: University Press.

IVERSEN, L.L. (1971). The uptake of biogenic amines. In *Biogenic Amines and Physiological Membranes and Drug Therapy*. (Part B). ed Biel, J.H. & Abood, L.G., New York: Marcel Dekker.

JASKI, B.E., WIJNS, W., FOULDS, R. & SERRUYS, P.W. (1986). The haemodynamic and myocardial effects of dopexamine: a new β_2-adrenoceptor and dopaminergic agonist. *Br. J. clin. Pharmac.*, **21**, 393–400.

OFFERMIER, J., SMIT, E.S. & DREYER, A.C. (1976). Assessment of two in vitro methods for determination of the apparent affinities of β-sympathomimetics on myocardial contractility. *J. Pharm. Pharmac.*, **28**, 787–788.

SMITH, G.W., HALL, J.C., FARMER, J.B. & SIMPSON, W.T. (1987). The cardiovascular actions of dopexamine hydrochloride, an agonist at dopamine receptors and β_2-adrenoceptors. *J. Pharm. Pharmac*, (in press).

SVENSSON, G., SJOGREN, A. & ERHARDT, L. (1986). Short-term haemodynamic effects of dopexamine in patients with chronic congestive heart failure. *Eur. Heart, J.*, **7**, 697–703.

WOOD, M.D. & WYLLIE, M.G. (1983). Critical assessment of noradrenaline uptake in synaptosomal preparations. *Naunyn Schmiedebergs Arch. Pharmac.*, **322**, 129–135.

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