Br. J. Pharmac. (1982), **75**, 617–621

PRESYNAPTIC EFFECT OF CLONIDINE ANTAGONIZED BY THE TETRAMINE DISULPHIDE, BENEXTRAMINE

B. BELLEAU, B.G. BENFEY* & C. MELCHIORRE[1]

Department of Chemistry and Department of Pharmacology and Therapeutics*, McGill University, Montreal, Quebec, Canada

1 The presynaptic α-adrenoceptor blocking activity of the newly synthesized α-adrenoceptor blocking drug, benextramine, was evaluated in the isolated left atrium of the guinea-pig heart.

2 High-voltage stimulation increased the force of contraction of electrically driven atrial strips, presumably by releasing noradrenaline from sympathetic nerve endings. Like phentolamine, benextramine increased the effect of high-voltage stimulation, presumably by blocking presynaptic α-adrenoceptors.

3 Clonidine reduced the effect of high-voltage stimulation, presumably by stimulating presynaptic α-adrenoceptors. The inhibitory effect of clonidine was antagonized noncompetitively by benextramine and competitively by phentolamine.

4 Combined administration of benextramine and phentolamine only resulted in the competitive phentolamine antagonism. Thus phentolamine protected the presynaptic α-adrenoceptors against benextramine blockade.

Introduction

The newly synthesized drug, benextramine, blocks postsynaptic α-adrenoceptors in the rabbit isolated aorta and rat isolated vas deferens non-competitively and irreversibly (Melchiorre, Yong, Benfey & Belleau, 1978). It was our intention to see whether benextramine also blocks the presynaptic α-adrenoceptors that modulate the release of noradrenaline from sympathetic nerve fibres in the heart. We used a simple method for stimulating autonomic nerves and causing the release of transmitters: subjecting isolated heart muscle preparations to intense electrical stimulation (Blinks, 1966; Katz & Kopin, 1969). In the rabbit isolated heart the sympathomimetic drug, clonidine, antagonized the increase in the stimulation-induced release of noradrenaline caused by the α-adrenoceptor blocking drug, phentolamine (Starke & Altmann, 1973). In the guinea-pig left atrial strip clonidine reduced, and phentolamine increased, the positive inotropic response to intense electrical stimulation (Malta, Ong, Raper, Tawa & Vaughan, 1980).

The experiments were carried out in the presence of cocaine because α-adrenoceptor blocking drugs can effect neuronal noradrenaline uptake. Thus phentolamine potentiated the inotropic and chronotropic effects of noradrenaline in the guinea-pig isolated atrium (Benfey & Greeff, 1961) and increased noradrenaline overflow in the rabbit isolated heart by inhibiting noradrenaline removal (Starke, Montel & Wagner, 1971). In the rabbit heart, cocaine (15 μM) did not inhibit the increase in noradrenaline caused by phentolamine (Starke *et al.*, 1971) but reduced the decrease in noradrenaline release caused by clonidine (Starke & Altmann, 1973). Clonidine must compete with endogenous noradrenaline for presynaptic α-adrenoceptors, and cocaine increases the concentration of noradrenaline in the synaptic cleft by inhibiting its neuronal uptake.

Methods

Strips, about 2 mm wide and 8 mm long, were cut from the outer wall of the left atrial appendage of the heart of immature guinea-pigs of either sex, weighing 150–170 g, and suspended at 37°C in a solution consisting of (mM): NaCl 114.9, $NaHCO_3$ 24.9, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, and glucose 10, which was aerated with 5% CO_2 in O_2. The resting tension of the strips was adjusted to approximately half the level associated with maximal developed tension. The tension developed in isometric contractions was recorded by means of force-displacement transducers (Grass FT .03C) and a polygraph (Grass). The strips were driven at a rate of 0.5 Hz through small platinum electrodes by square

[1]Present address: Istituto di Chimica Farmaceutica e di Chimica Organica, Universita di Camerino, 62032 Camerino, Italy.

0007-1188/82/040617-05 $01.00

wave pulses of 1 ms duration and of an intensity, determined in each preparation, to be just enough to elicit contractions, generally 1 V, using a Grass stimulator. A ten fold increase in the voltage every 10 min for a period of 40 s produced submaximal positive inotropic responses which remained constant for several hours.

Several control responses to sympathetic stimulation were first obtained with each preparation and then increasing concentrations of the drugs were added to the bathing solution. The concentrations of clonidine were increased in 10 min intervals, those of phentolamine in 20 min intervals, and those of benextramine in 40 min intervals. Longer time intervals did not lead to greater effects.

Atropine (2 μM) was added before the start of the experiments to inhibit the effect of acetylcholine released from parasympathetic nerve endings. Cocaine (20 μM) was added before the start of the experiment to prevent an effect of the α-adrenoceptor blocking drugs on neuronal noradrenaline uptake. In the presence of cocaine (20 μM) the inotropic effect of a small concentration of noradrenaline (4 nM) was neither potentiated by phentolamine (0.1–3 μM) or benextramine (0.3–30 μM) nor inhibited by clonidine (0.01–30 μM).

The following drugs were used: atropine sulphate (Merck), benextramine (N,N′-bis(6-[*o*-methoxybenzylamino]-*n*-hexyl)cystamine tetrahydrochloride monohydrate, Aldrich), clonidine hydrochloride (Boehringer, Ingelheim), cocaine hydrochloride (Merck), (−)-noradrenaline bitartrate (Winthrop), and phentolamine methane sulphonate (Ciba).

Statistical calculations were performed according to conventional procedures (Snedecor & Cochran, 1967).

Results

Figure 1 shows that phentolamine and benextramine had a small negative inotropic effect on atrial contractions stimulated with threshold shocks and increased the positive inotropic effect of intense electrical stimulation. The EC_{50} (mean ± s.e.) of phentolamine was 0.10 ± 0.023 μM (8 experiments) and that of benextramine was 2.3 ± 0.65 μM (12 experiments).

Figure 2 shows that clonidine reduced the response to strong electrical stimulation and that phentolamine shifted the clonidine dose-response curve to the right. The EC_{50} of clonidine was 0.22 μM before, and 3.0 μM after the addition of 1.4 μM phentolamine; thus the dose-ratio was 13.6 and the K_B of phentolamine ([phentolamine]/dose-ratio − 1; mean ± s.e. of 4 experiments) was 0.11 ± 0.025 μM. Thus phentolamine increased the effect of electrical

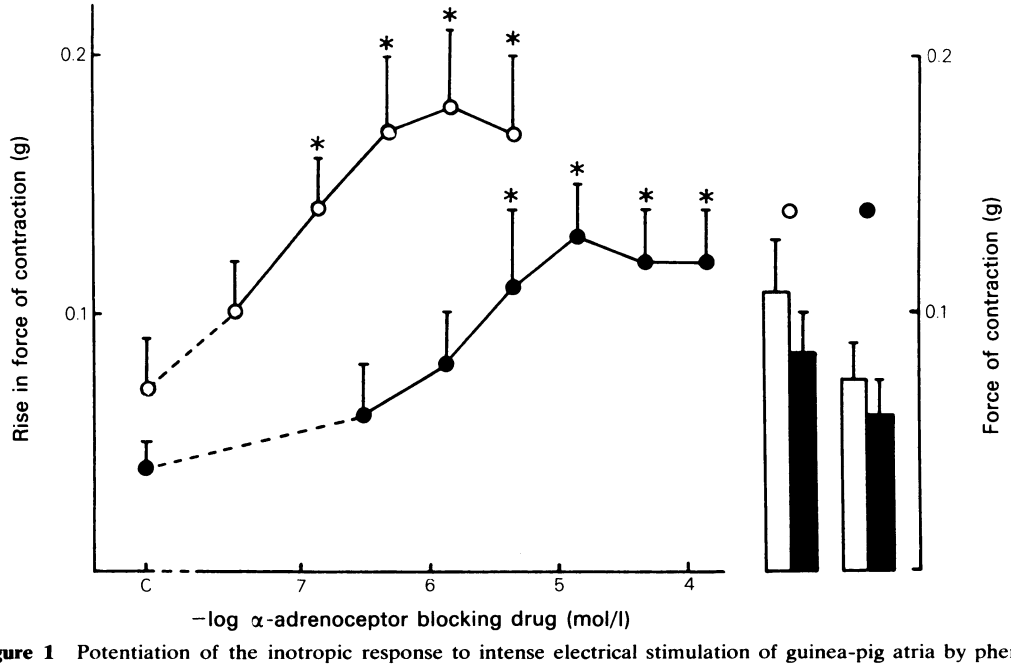

Figure 1 Potentiation of the inotropic response to intense electrical stimulation of guinea-pig atria by phentolamine (○, means of 8 experiments) and benextramine (●, means of 12 experiments). The vertical bars represent s.e.means. *$P < 0.05$, compared with the controls (C). The open columns represent the initial force of contraction and the solid columns the force of contraction in the presence of the highest drug concentration.

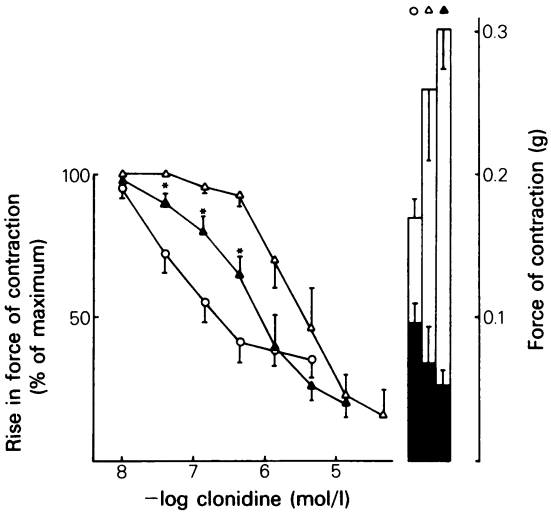

Figure 2 Inhibition by clonidine of the inotropic response to strong electrical stimulation of guinea-pig atria in the absence (○, means of 12 experiments) and presence of phentolamine 1.4 μM (△, means of 4 experiments), and 20 min after removal of phentolamine (1.4 μM) and repeated changes of the bath fluid (▲, means of 4 experiments). The vertical bars represent s.e.means. $*P<0.05$, when compared with the clonidine effect in the presence of phentolamine. The solid columns represent the initial force of contraction and the open columns the inotropic effect of high-voltage stimulation before adding clonidine.

stimulation and reduced the effect of clonidine with a similar potency. Twenty minutes after the withdrawal of phentolamine and following repeated changes of the bath fluid the potency of clonidine was increased; thus the effect of phentolamine was slowly reversible.

Figure 3 shows that 1 μM benextramine slightly shifted the clonidine dose-response curve to the right (the EC_{50} of clonidine was 0.56 μM) and that 14 μM benextramine antagonized the clonidine effect non-competitively. Forty minutes after the withdrawal of 14 μM benextramine and following repeated changes of the bath fluid the potency of clonidine was increased; thus the effect of benextramine appeared to be slowly reversible.

Figure 4 shows that benextramine and phentolamine have the same site of action. The effect of 3 μM benextramine was similar to that of 14 μM benextramine; the simultaneous administration of 3 μM benextramine and 1 μM phentolamine eliminated the noncompetitive benextramine blockade and only led to the competitive phentolamine blockade.

Discussion

From the results of studies in the rabbit isolated heart (Starke *et al.*, 1971) we assume that phentolamine increases the inotropic effect of strong electrical stimulation (Figure 1) by blocking presynaptic α-adrenoceptors which augments the release of noradrenaline. Benextramine appears to have the same effect. Clonidine is a partial agonist on cardiac presynaptic α-adrenoceptors (Werner, Starke & Schümann, 1972; Starke & Altmann, 1973; Medgett, McCulloch & Rand, 1978; Malta *et al.*, 1980; and Figure 2 of this study).

In the rabbit isolated left atrium, phentolamine (K_B 31 nM) and benextramine (IC_{50} 0.26 μM) antagonized the positive inotropic effect of phenylephrine (Benfey, Belleau, Brasili, Giannella & Melchiorre, 1980). Thus the presynaptic α-adrenoceptor blocking potency of phentolamine (EC_{50} 0.10 μM) was 3.2 times lower than its postsynaptic α-adrenoceptor blocking potency, and the presynaptic α-adrenoceptor blocking potency of benextramine (EC_{50} 2.3 μM) was 8.9 times lower than its postsynaptic α-adrenoceptor blocking potency. But it is difficult to determine the potency of agents acting on presynaptic α-adrenoceptors accurately because the amount of endogenous noradrenaline in the synaptic cleft which competes for the receptors can vary widely. Thus in the rabbit heart the degree of inhibition by clonidine of noradrenaline release during sympathetic nerve stimulation was inversely related to the release of noradrenaline in response to stimulation before administration of clonidine (Starke & Altmann, 1973).

Phentolamine blocks α-adrenoceptors reversibly and competitively, and in the rabbit heart the effect of phentolamine on noradrenaline release was slowly reversible (Starke *et al.*, 1971). Figure 2 shows that 20 min after the removal of phentolamine the clonidine effect recovered significantly. Benextramine blocks α-adrenoceptors irreversibly and noncompetitively (Melchiorre *et al.*, 1978; Benfey *et al.*, 1980). Figure 3 shows that a partial recovery of the clonidine effect appeared to occur 40 min after the removal of benextramine and that benextramine acted noncompetitively.

Proof that benextramine and phentolamine act at the same site was obtained when 3 μM benextramine and 1 μM phentolamine were administered together: there was only the competitive phentolamine blockade (Figure 4). A similar phenomenon was described by Benfey & Grillo (1963). In the guinea-pig isolated atrium the phenoxybenzamine blockade of the negative chronotropic effect of acetylcholine was slow in onset and offset and the atropine blockade had a fast onset and offset. When 0.47 μM phenoxybenzamine was given together with 0.1 μM atropine, the slow,

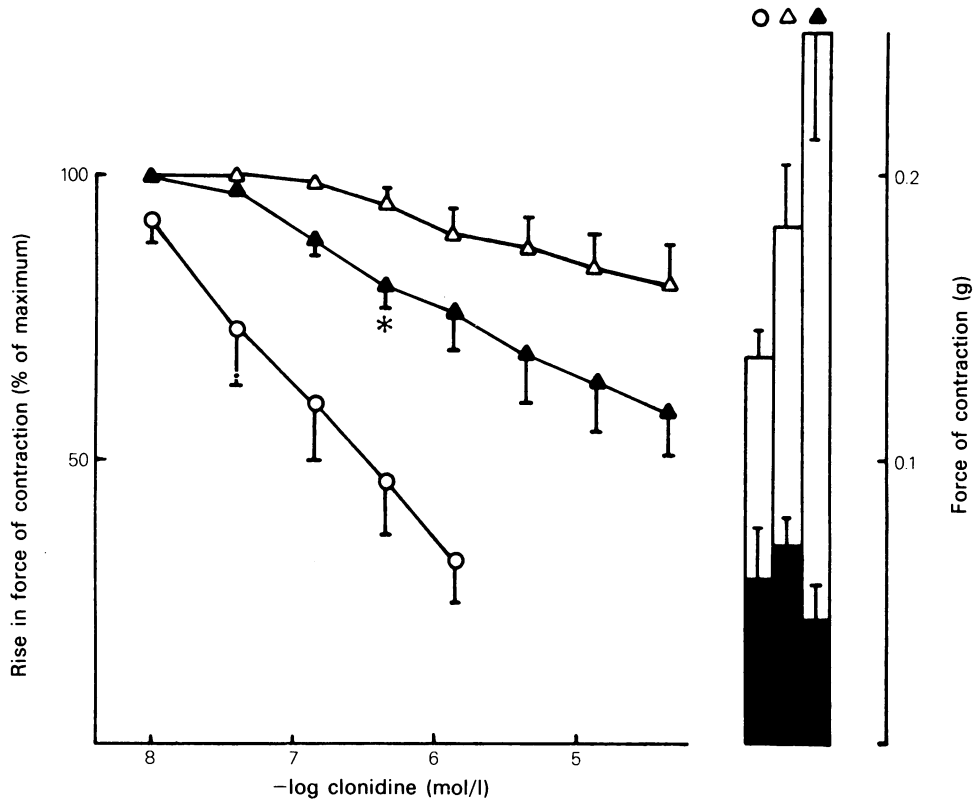

Figure 3 Inhibition by clonidine of the inotropic response to strong electrical stimulation of guinea-pig atria in the presence of 1 μM benextramine (○, means of 4 experiments), 14 μM benextramine (△, means of 8 experiments), and 40 min after removal of 14 μM benextramine and repeated changes of the bath fluid (▲, means of 8 experiments). The vertical bars represent s.e.means. $*P<0.05$, compared with the clonidine effect in the presence of benextramine. The solid columns represent the initial force of contraction and the open columns the inotropic effect of high-voltage stimulation before adding clonidine.

irreversible phenoxybenzamine blockade disappeared and only the fast, reversible atropine blockade remained. Thus atropine protected the muscarinic receptor against phenoxybenzamine blockade.

In conclusion, we have shown that, by blocking presynaptic α-adrenoceptors, benextramine potentiates the inotropic effect of intense electrical stimulation and antagonizes the inhibition by clonidine of the inotropic effect of electrical stimulation.

This study was supported by a grant from the Medical Research Council of Canada. Reprint requests to B.G.B., Department of Pharmacology and Therapeutics, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6.

References

BENFEY, B.G., BELLEAU, B., BRASILI, L., GIANNELLA, M. & MELCHIORRE, C. (1980). Irreversible α-adrenoceptor blocking effects and reversible muscarinic blocking and nicotinic blocking effects of tetramine disulfides in the heart. *Can. J. Physiol. Pharmac.*, **58**, 1477–1483.

BENFEY, B.G. & GREEFF, K. (1961). Interaction of sympathomimetic drugs and their antagonists on the isolated atrium. *Br. J. Pharmac. Chemother.*, **17**, 232–235.

BENFEY, B.G. & GRILLO, S.A. (1963). Antagonism of acetylcholine by adrenaline antagonists. *Br. J. Pharmac. Chemother.*, **20**, 528–533.

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.

KATZ, R.I. & KOPIN, I.J. (1969). Electrical field-stimulated release of norepinephrine-H^3 from rat atrium: effects of

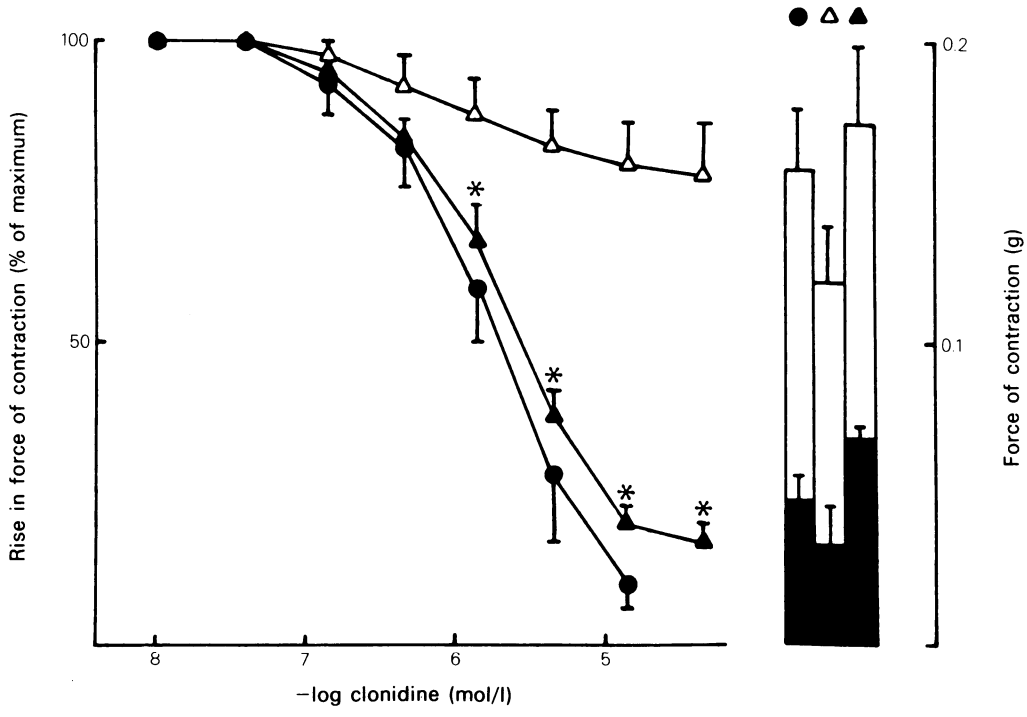

Figure 4 Inhibition by clonidine of the inotropic response to strong electrical stimulation of guinea-pig atria in the presence of phentolamine 1 μM (●, means of 4 experiments), benextramine 3 μM (△, means of 4 experiments), and phentolamine (1 μM) plus benextramine (3 μM) (▲, means of 4 experiments). The vertical bars represent s.e.means. *$P<0.05$, compared with the clonidine effect in the presence of benextramine (3 μM) alone. The solid columns represent the initial force of contraction and the open columns the inotropic effect of high-voltage stimulation before adding clonidine.

ions and drugs. *J. Pharmac. exp. Ther.*, **169,** 229–236.

MALTA, E., ONG, J.S.B., RAPER, C., TAWA, P.E. & VAUGHAN, G.N. (1980). Structure-activity relationships of clonidine- and tolazoline-like compounds at histamine and α-adrenoceptor sites. *Br. J. Pharmac.*, **69,** 679–688.

MEDGETT, I.C., MCCULLOCH, M.W. & RAND, M.J. (1978). Partial agonist action of clonidine on prejunctional and postjunctional α-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmac.*, **304,** 215–221.

MELCHIORRE, C., YONG, M.S., BENFEY, B.G. & BELLEAU, B. (1978). Molecular properties of the adrenergic α-receptor. 2. Optimum covalent inhibition by two different prototypes of polyamine disulfides. *J. med. Chem.*, **21,** 1126–1132.

SNEDECOR, G.M. & COCHRAN, W.G. (1967). *Statistical Methods.* Ames, Iowa: Iowa State University Press.

STARKE, K. & ALTMANN, K.P. (1973). Inhibition of adrenergic neurotransmission by clonidine: an action on prejunctional α-receptors. *Neuropharmac.*, **12,** 339–347.

STARKE, K., MONTEL, H. & WAGNER, J. (1971). Effect of phentolamine on noradrenaline uptake and release. *Naunyn-Schmiedebergs Arch. Pharmac.*, **271,** 181–192.

WERNER, U., STARKE, K. & SCHUMANN, H.J. (1972). Actions of clonidine and 2-(2-methyl-6-ethylcyclohexylamino)-2-oxazoline on postganglionic autonomic nerves. *Archs. int. Pharmacodyn.*, **195,** 282–290.

(Received October 13, 1981.
Revised November 23, 1981.)