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INHIBITORY EFFECTS OF CLONIDINE AND BS 100-141 ON RESPONSES TO SYMPATHETIC NERVE STIMULATION IN CATS AND RABBITS

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1 In pithed cats, the spinal sympathetic outflow was stimulated preganglionically at segments C7 and T1 and heart rate responses and nictitating membrane tone were measured in parallel.

2 Clonidine and a related drug, BS 100-141 (N-amidino-2-(2,6-dichlorophenyl)acetamide hydrochloride), caused a dose-dependent inhibition of the stimulation-induced tachycardia but did not inhibit responses of the nictitating membrane. The inhibition of heart rate was antagonized by the α-adrenoceptor blocking drug, phentolamine.

3 In isolated hearts of rabbits, noradrenaline release in response to adrenergic nerve stimulation was reduced by clonidine and BS 100-141 and the effect was antagonized by phentolamine.

4 The results support the view that presynaptic α-adrenoceptors are involved in the regulation of transmitter release from adrenergic nerves. Cardiac adrenergic nerves appear more sensitive to α-adrenoceptor-mediated inhibition of impulse transmission than the sympathetic nerves to the nictitating membrane.

Introduction

Clonidine inhibits the effect of sympathetic stimulation on the heart in anaesthetized cats (Kobinger, 1967), pithed rats (Armstrong & Boura, 1973) and isolated hearts of rabbits (Starke & Altmann, 1973). Starke & Altmann (1973) have suggested that this effect is probably due to stimulation of α-adrenoceptors on postganglionic sympathetic nerve endings, which modulate transmitter release. That post-ganglionic nerves are endowed with α-adrenoceptors, stimulation of which causes a decrease and blockade an increase of transmitter release, has been demonstrated by Kirpekar & Puig (1971).

In the present paper, we have further tested the hypothesis that clonidine has an action on presynaptic α-adrenoceptors and have carried out comparative studies using a compound with similar effects, namely, N-amidino-2-(2,6-dichlorophenyl)-acetamide hydrochloride (BS 100-141) (Figure 1). The spinal sympathetic outflow in pithed cats was electrically stimulated and the inhibitory effects of these compounds on the heart rate and their antagonism by phentolamine were investigated. In other experiments the effects of these compounds on the output of noradrenaline in sympathetically stimulated rabbit hearts were measured. In the cat experiments, effects on the responses of the nictitating membrane to sympathetic nerve stimulation were examined.

Methods

Cats of either sex, 2-3 kg, were anaesthetized with pentobarbitone sodium (40 mg/kg i.p.) and pithed (Gillespie, Maclaren & Pollock, 1970) after bilateral cervical vagotomy. Artificial respiration was applied and an intravenous infusion of

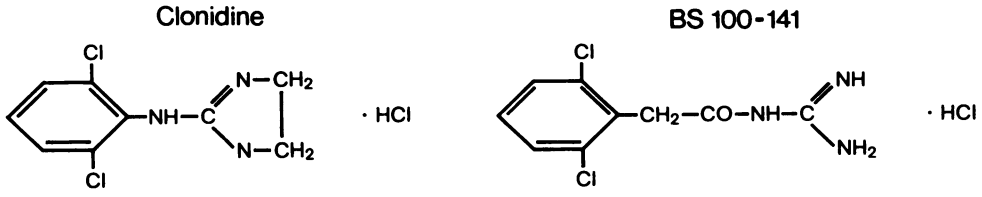

Figure 1

suxamethonium, 50 μg kg^{-1} min^{-1}, was given during the whole experiment. Body temperature was maintained at 37° C. Femoral arterial blood pressure was measured by means of a Statham P23 Db transducer. Integrated heart rate was recorded by a cardiotachometer. The left nictitating membrane was connected to a strain gauge transducer and pre-loaded with approximately 2 grams. Blood pressure, heart rate and isometric contractions of the nictitating membrane were recorded by means of an Offner S-II polygraph.

A pithing rod was used for stimulation of the spinal sympathetic outflow. The rod consisted of three sections: the cranial part (29 cm) and the caudal part (20 cm), both made of Perspex and connected to a short central section (2 cm) made of stainless steel. The rod had a uniform outer diameter of 4 mm. The middle part, used as the stimulation electrode, was placed, under X-ray monitoring, close to the spinal segments C7 and T1. The indifferent electrode was placed subcutaneously on the back.

The following stimulation procedure was adopted. Trains of supramaximal shocks (50 V) of 1 ms duration were applied for periods of 5 or 10 seconds. Each train of stimuli was followed by a 5 min interval, after which another stimulus train at the next higher frequency was applied. The initial stimulation frequency was usually 1 Hz and this was successively doubled until a frequency of 8 Hz was reached. Stimulation caused a transient increase in the heart rate which was followed by complete recovery to the original base line during intervals. The increase in heart rate was graded according to the stimulation frequency as shown in Figure 2. In 10 control experiments, stimulation with 0.5, 1, 2, 4, 8 and 16 Hz produced mean increases in heart rate of 5, 13, 28, 44, 58 and 67 beats/min respectively. Figure 2 also shows graded contractions of the nictitating membrane that depend on stimulation frequency.

Rabbit isolated hearts were perfused via the coronary vessels with Tyrode solution and their postganglionic sympathetic nerves stimulated (Huković & Muscholl, 1962). Two or three stimulation periods of 1 min duration (50 V, 3 ms, 7 Hz) were applied in each experiment. The time interval between successive stimulation periods was 15 minutes.

Perfusate collection lasted for 2 min from the onset of electrical stimulation. In the experiments of Table 1, drugs were infused from 10 min before the start of the second stimulation period. In those of Table 2, BS 100-141 was added 10 min before the start of the second stimulation period and subsequently left in the perfusate. Phentolamine was added 10 min before the start of the third stimulation period and subsequently left in the perfusate. Noradrenaline levels were determined fluorimetrically (Anton & Sayre, 1962).

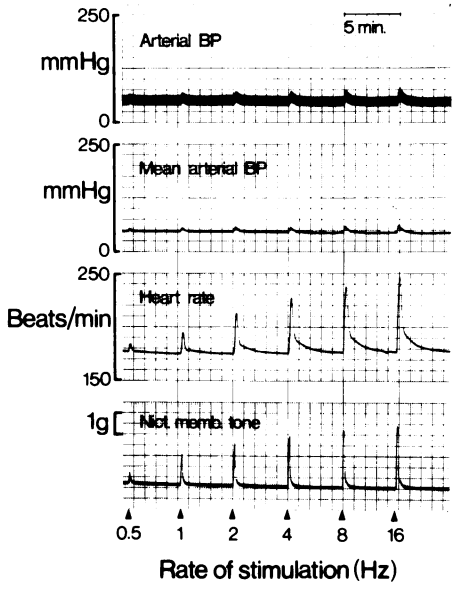

Figure 2 Stimulation of the spinal sympathetic outflow at segments C7 and T1 in a pithed cat. Stimulation parameters: 50 V, 1 ms during 10 s, with frequencies indicated at arrows. The tracings show effects on (from top) pulsatile femoral arterial blood pressure, mean blood pressure, heart rate and nictitating membrane tone (isometric recording).

Results

Heart rate in cats

Clonidine or BS 100-141 administered intravenously in doses of 5 and 15 μg/kg 5 min before beginning electrical stimulation of spinal segments C7 and T1 did not affect the resting rate but antagonized stimulation-induced increases in heart rate. As shown in Figure 3, the stimulation frequency-response curves were displaced to the right in a dose-dependent manner. The displacement occurred with both clonidine and BS 100-141 but was greater with the former drug. Within the range of stimulation frequencies employed, the stimulation frequency-response curves remained approximately parallel.

These effects of clonidine and BS 100-141 on the heart rate responses persisted for several hours but when the α-adrenoceptor blocking drug phentolamine (1 mg/kg) was administered 20 min after the higher dose of clonidine or BS 100-141,

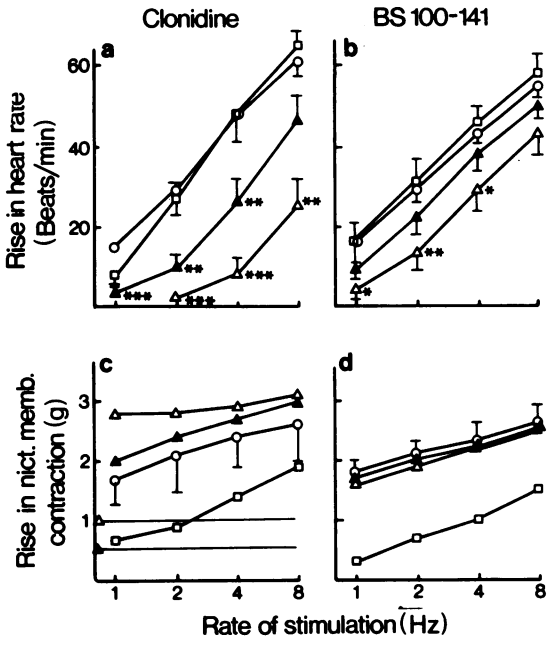

Figure 3 Responses of heart rate (a, b) and nictitating membrane (c, d) to stimulation of the spinal sympathetic outflow in pithed cats before (○) and after 5 μg/kg (▲) and 15 μg/kg i.v. (△) of clonidine or BS 100-141 and after a subsequent injection of phentolamine (1 mg/kg i.v.) (□). The time interval between drug injections was 20 minutes. The horizontal lines at the bottom of section (c) indicate the rise of basic tone in the nictitating membrane obtained with clonidine 5 μg/kg (▲) and 15 μg/kg i.v. (△) in the absence of nerve stimulation. Values are the mean for five cats. Vertical lines show s.e. mean. Significant difference from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's t test).

their effects on heart rate were completely abolished (Figure 3a, b).

Contraction of the nictitating membrane

BS 100-141 had no appreciable contractile effect of its own and did not antagonize significantly the contractile effects of electrical stimulation of the nictitating membrane (Figure 3d) at doses which clearly antagonized sympathetic stimulation of the heart rate. Clonidine had a definite contractile effect of its own on the nictitating membrane so that the contractile effects of sympathetic stimulation were superimposed on an already raised base line (Figure 3c). Sympathetic stimulation in the presence of clonidine produced larger contractions than in its absence and although the results are not strictly comparable owing to the differences in base line there was no evidence that clonidine antagonized the effects of sympathetic stimulation on the nictitating membrane as it did in the heart.

As expected from an α-adrenoceptor blocking agent, phentolamine antagonized stimulation responses of the nictitating membrane (Figure 3c, d).

Noradrenaline output in rabbit hearts

In rabbit isolated hearts the release of noradrenaline following sympathetic nerve stimulation was significantly reduced by clonidine and BS 100-141 (Table. 1). In a further set of experiments (Table 2), phentolamine as well as BS 100-141 was used. BS 100-141 was perfused during the second and third stimulation periods and phentolamine during the third stimulation period. BS 100-141 inhibited noradrenaline output and phentolamine reversed the inhibition.

Table 1 Effects of BS 100-141 and clonidine on the output of noradrenaline (NA) during sympathetic nerve stimulation in rabbit isolated hearts

Drug	*Drug concentration (μg/ml during S_2)*	*NA output (ng per stimulation period)* S_1	S_2	n
Control	0	53 ± 6	51 ± 9	20
BS 100-141	0.04	41 ± 8	22 ± 5*	4
	0.4	42 ± 7	16 ± 3**	4
Clonidine	0.004	66 ± 10	42 ± 13*	8
	0.04	59 ± 9	34 ± 5**	6
	0.4	43 ± 8	15 ± 3**	6

The outputs S_1 and S_2 are prior to and in the presence of drug respectively. Mean values ± s.e. mean. n = number of experiments.
* $P < 0.05$; ** $P < 0.01$; t values were calculated by comparing S_1–S_2 in controls and after drug administration

Table 2 Effects of BS 100-141 (0.04 μg/ml) and phentolamine (0.12 μg/ml) on noradrenaline (NA) output during sympathetic nerve stimulation in rabbit isolated hearts

NA output (ng per stimulation period)		
S_1	S_2	S_3
Control	*BS 100-141*	
53 ± 7	22 ± 4	16 ± 4
		+ Phentolamine
59 ± 7	29 ± 4	69 ± 18**

Means of five determinations ± s.e. mean. S_1, S_2 and S_3 are successive stimulation periods.
** $P < 0.01$ for values of S_3–S_2 with and without phentolamine.

Discussion

The experiments show that the effect of clonidine on the heart has a peripheral component in addition to its known central component (Schmitt, Schmitt & Fenard, 1971). Similar peripheral effects were shown by both clonidine and BS 100-141 and they can be explained by assuming that these drugs stimulate presynaptic α-adrenoceptors that inhibit transmitter release as postulated by Kirpekar & Puig (1971). In favour of this explanation is the finding that both drugs produced a dose-dependent inhibition of sympathetic cardioacceleration which could be antagonized by phentolamine. The experiments on the sympathetically stimulated rabbit heart provided direct evidence of inhibition of noradrenaline release by clonidine confirming Starke & Altmann (1973). Our experiments show that BS 100-141 also inhibits stimulation-induced noradrenaline release and that this effect is antagonized by phentolamine.

Inhibitory effects of clonidine on sympathetic cardioacceleration had previously been demonstrated by Kobinger (1967) and Armstrong & Boura (1973), both finding that the inhibitory effects occurred preferentially at low stimulation frequencies. Our own experiments showed no evidence of preferential inhibition between the frequencies of 1 to 8 Hz. Possibly the discrepancy is due to differences in stimulation procedure, such as the very short stimulation periods used in the present work.

Our results indicate a certain cardioselectivity of clonidine-like α-adrenoceptor stimulant drugs. Doses of clonidine and BS 100-141 which produced a marked inhibition of the effect of stimulation of the sympathetic outflow to the heart did not influence the maximum tension developed in the nictitating membrane during stimulation of its sympathetic outflow. Guanabenz shows similar effects (Scholtysik, 1974). The cause of this cardioselective adrenergic neurone inhibition remains unknown.

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