Br. J. Pharmac. (1975), 54, 17-21

EFFECT OF CLONIDINE ON THE EXCITABILITY OF VASOMOTOR LOCI IN THE CAT

B.N. DHAWAN, M.B. JOHRI,[1] G.B. SINGH, R.C. SRIMAL & D. VISWESARAM[2]
Division of Pharmacology, Central Drug Research Institute, Lucknow-1, India

1 The effect of clonidine on the direct excitability of hypothalamic, medullary and spinal vasomotor loci has been investigated in cats anaesthetized with chloralose.

2 Clonidine inhibited the excitability of these loci when it was localized to the central sites by intracerebroventricular, intravertebral arterial or intrathecal injection in very low doses (1-2 μg).

3 Topical application of clonidine (0.01% and 1.0%) to the floor of the fourth ventricle inhibited pressor responses evoked either by stimulation of medullary or hypothalamic vasomotor areas. Inhibition of the pressor responses was accompanied by hypotension and bradycardia in many experiments.

4 It appears that effects of clonidine on the vasomotor loci of the medulla oblongata and the spinal cord contribute to its hypotensive action.

Introduction

Intravenous administration of clonidine (Catapres) produces initial sympathomimetic effects by stimulating α-adrenoceptors (Maling, Cho, Horakova & Williams, 1969) followed by hypotension and bradycardia which have been ascribed to a central action (Sherman, Greca, Woods & Buckley, 1968; Shaw, Hunyor & Korner, 1971). Hukuhara, Otsuka, Tadeka & Sakai (1968) and Schmitt & Schmitt (1969) reported an inhibition of the action potentials in the splanchnic and cardiac nerves following central administration of clonidine. They localized the site of this action of clonidine to the medulla oblongata. Recently Boudier & Van Rossum (1972) reported that the injection of clonidine into the rat hypothalamus produced hypotension. The present study was undertaken to obtain direct evidence of the effect of clonidine on the excitability of the hypothalamic, medullary and spinal vasomotor loci.

Methods

All experiments were performed in adult cats (2.5-4.0 kg) of either sex. They were anaesthetized with chloralose (80.0 mg/kg, i.v.), vagotomized and put on artificial positive pressure respiration as a routine. One of the femoral veins was cannulated with a polythene tube. Blood pressure was recorded from the left femoral artery either through a Statham P23dc pressure transducer on a Grass Model 7 polygraph or through a mercury manometer on a kymograph. In some experiments heart rate was also recorded through a Grass 7P4 tachograph preamplifier.

Effect on hypothalamic and medullary vasomotor loci

Hypothalamic and medullary vasomotor loci were stimulated electrically (0.5-4 V, 1 ms, 100 Hz for 5 s) by means of stereotaxically placed bipolar concentric stainless steel needle electrodes insulated except for 0.5 mm at their tips. Placement of the electrodes was aided by the coordinates given by Jasper & Ajmone-Marson (1954) as follows: for hypothalamus, frontal 9-10 mm, lateral 1-2 mm and depth 4-6 mm; for medulla, caudal 8-10 mm, lateral 2-4 mm and depth 4-6 mm. The depth was fixed by repeated stimulation after slowly driving the electrode into the tissue and choosing the most reactive point. Square wave pulses were derived from an Aplab electronic stimulator. A series of voltages were used to obtain graded vasomotor responses. The smallest voltage giving a clearcut response was called a threshold response while a larger submaximal response was called an optimal response. The drug was not given until these control responses were constant.

Clonidine was administered into the left lateral ventricle, through a Collison cannula implanted

[1] Present address: Department of Pharmacology, A.M.U. Medical College, Aligarh, India.
[2] Present address: Department of Pharmacy, Andhra University, Visakapatnam, India.

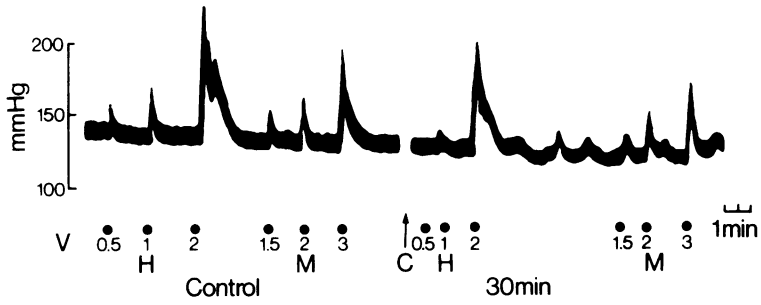

Figure 1 Blood pressure of a 3 kg cat anaesthetized with chloralose. Pressor responses to 0.5 (14 mmHg), 1.0 (34 mmHg) and 2.0 V (88 mmHg) stimulation of hypothalamic (H) and 1.5 (20 mmHg), 2.0 (32 mmHg) and 3.0 V (66 mmHg) stimulation of medullary (M) vasomotor areas. Clonidine (1 μg i.c.v.) was administered at C. Note the inhibition of hypothalamic responses to 0, 11 and 75 mmHg respectively and the medullary vasomotor responses to 14, 30 and 52 mmHg respectively at 30 minutes. There is no significant change in the blood pressure level.

according to the technique of Feldberg & Sherwood (1953), in a volume of 0.15 ml followed by 0.10 ml of 0.9% w/v NaCl solution (saline).

In some cats the drug was administered into a vertebral artery by means of a polythene tube introduced through the brachial artery with its tip close to the origin of the verebral artery. All branches of the subclavian artery except the common carotid were ligated. At the time of injection of the drug the common carotid artery was temporarily occluded.

The effect of the topical application of the drug to the floor of the IVth ventricle on the medullary and hypothalamic responses was studied in other cats. Part of the occipital bone was removed and the dura was cut away. The cerebellum was then pushed upwards to expose the floor of the fourth ventricle. Medullary and hypothalamic loci were electrically stimulated as described above and a cotton pledget soaked in the drug solution was applied at the site of the medullary electrode.

Effect on spinal vasomotor loci

The effect of the drug on the excitability of the spinal vasomotor neurones was assessed by eliciting spinal compression vasomotor responses (SCVR). The spinal cord was ligated within the meninges at C-7. A hypodermic needle, attached to a pressure bottle filled with saline for transmitting fluid compression to spinal cord and connected in parallel with an aneroid manometer, was introduced into the theca at the lumbosacral articulation. Spinal compression (100-150 mmHg) for 10-20 s elicited a consistent pressor response (White & Borison, 1955). The drug was injected intrathecally in a volume of 0.2 ml followed by 0.1 ml of saline through the same needle which was used for spinal compression.

Whenever possible, mean alteration from control responses has been calculated and the standard error of the mean (s.e.) determined. The values shown in the results are mean values ± s.e.

Results

Effect of intracerebroventricular administration

Intracerebroventricular injection of 1-2 μg clonidine in 10 cats inhibited the hypothalamic as well as medullary vasomotor responses for 90-120 minutes. With an intracerebroventricular dose of 1 μg the hypothalamic threshold vasomotor response was inhibited by 41 ± 16.3% whereas an optimal vasomotor response was inhibited by 44 ± 12.3%. The same dose of clonidine inhibited the medullary threshold response by 50 ± 12.3% and the optimal response by 36 ± 8.3%. The inhibitory effect started in 5-10 min and reached a peak in 20-40 minutes. Inhibition of the vasomotor responses was accompanied by a mild hypotension (8.0 ± 7.9 mmHg) (Figure 1).

Effect of intravertebral arterial injection

Intravertebral arterial injection of clonidine in 6 cats (1-2 μg) produced essentially the same effect as produced by intracerebroventricular administration. Drug administration was followed within 5-10 min by inhibition of hypothalamic as well as medullary pressor responses and bradycardia. The effect of the compound lasted 20-60 minutes. In

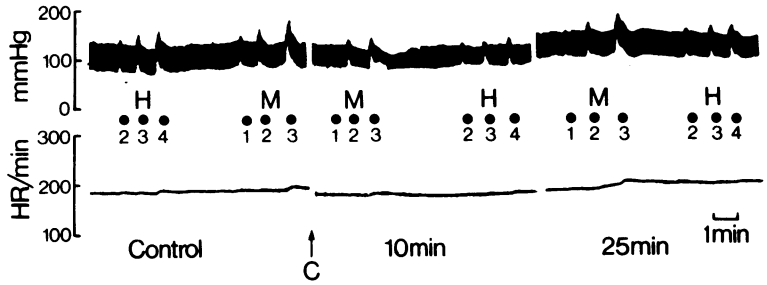

Figure 2 Blood pressure (mmHg) and heart rate (HR/min) of a 2.8 kg cat anaesthetized with chloralose. Hypothalamic (H) and medullary (M) vasomotor areas were stimulated by the voltages indicated at various points in the figure. Clonidine (2 μg) was injected into a vertebral artery at C. Blood pressure was lowered by 15 mmHg, bradycardia (20/min) occurred and responses were inhibited to hypothalamic stimulation (from 12, 20 and 35 to 8, 12 and 16 mmHg) and medullary stimulation (from 10, 30 and 45 to 8, 12 and 16 mmHg) at 10 min (middle panels). Partial recovery occurred after 25 min (last panels).

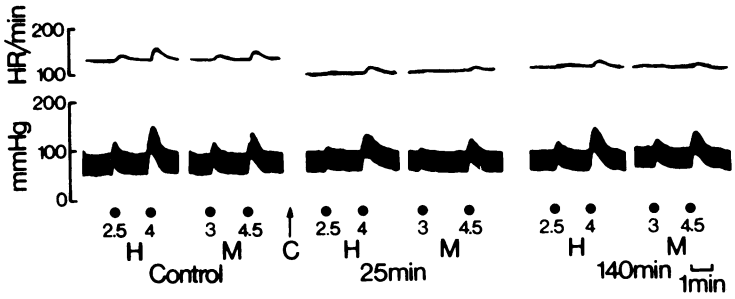

Figure 3 Heart rate (HR/min) and blood pressure (mmHg) of a 3 kg cat anaesthetized with chloralose. Threshold and optimal vasomotor responses to stimulation of hypothalamic (H) and medullary (M) vasomotor areas at the voltages indicated in the figure are shown. Clonidine 0.01% (C) was applied topically to the floor of the IVth ventricle. Note bradycardia (30/min) and inhibition of all the responses (hypothalamic pressor responses decreased from 30 and 55 to 10 and 35 mmHg and medullary pressure responses decreased from 25 and 40 to 5 and 25 mmHg respectively) at 25 min (middle panel). There was partial recovery after 140 minutes.

some experiments a weak hypotensive effect was also observed (Figure 2).

Effect of topical application

Topical application of clonidine (0.01% solution) to the floor of the fourth ventricle was followed by inhibition of threshold as well as optimal medullary and hypothalamic pressor responses within 10-20 min in 15 cats. Bradycardia also occurred. The medullary threshold pressor response was inhibited by 67 ± 16.2% whereas optimal pressor response was inhibited by 50 ± 9.2%. An inhibition (71 ± 6.5% and 62 ± 9% respectively) of hypothalamic pressor responses was also observed. Inhibition of responses was not accompanied by any significant hypotension in most of the cats (Figure 3). Recovery of the vasomotor responses occurred in 140 to 200 min after the removal of the drug-soaked cotton pledget. Topical application of a concentrated (1.0%) solution of clonidine produced hypotension (23 ± 11.9 mmHg) in addition to the inhibition of the medullary threshold (69 ± 12.9%) and optimal (53 ± 13.3%) vasomotor responses. The hypothalamic threshold and optimal vasomotor responses were inhibited by 95 ± 8.2% and 88 ± 11% respectively. With the higher concentration of clonidine the inhibition of the responses was obtained earlier (5-10 min) and persisted much longer (more than 180 minutes).

Effect on spinal vasomotor neurones

Intrathecal injection of 1-2 μg clonidine inhibited threshold as well as optimal SCVR in 7 cats. The threshold and optimal responses were inhibited by 44.5 ± 7.2% and 46 ± 8.6% respectively. Peak

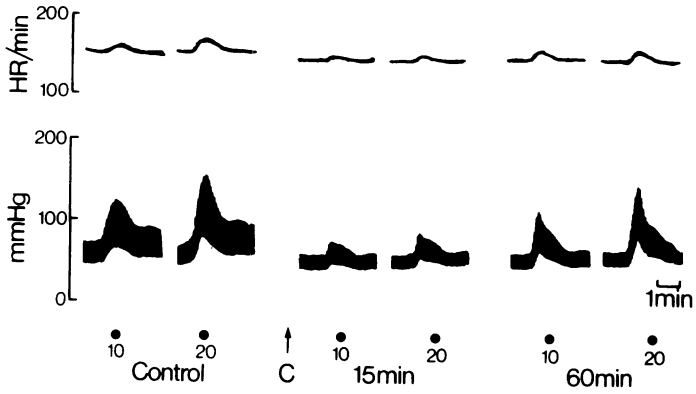

Figure 4 Heart rate (HR/min) and blood pressure (mmHg) of a 3.2 kg spinal transected cat. Spinal compression vasomotor responses (SCVR) were elicited by a 10 and 20 s compression. Clonidine, 1 μg (C) inhibited SCVR at 15 min from 65 and 85 to 25 and 35 mmHg respectively (middle panel). Recovery occurred at 60 min (last panel).

effect was evident in 5-15 min and recovery occurred in 60-120 minutes. There was no significant change in the blood pressure (Figure 4).

Discussion

Several authors have suggested a central mechanism in the hypotensive action of clonidine (Sherman *et al.*, 1968; Schmitt & Schmitt, 1969; Shaw *et al.*, 1971). However, their studies do not provide adequate information about the direct effects of clonidine on the excitability of neurones in central cardiovascular loci. In the present investigation the effect of clonidine on the excitability of these neurones, as judged by responses to electrical stimulation (hypothalamus, medulla) or pressure (spinal cord), has been studied because these stimuli give reproducible blood pressure and heart rate changes for several hours (Gupta, Srimal & Dhawan, 1972).

The present findings demonstrate an inhibitory effect of clonidine on the excitability of central vasomotor loci. The inhibitory action seems to be mainly exerted at the medullary and the spinal levels. An action at the medullary level has also been proposed by Schmitt & Schmitt (1969). The spinal cord is being increasingly recognized as a possible central site for cardiovascular effects (Smith, 1974) and it is interesting to note that the spinal loci are also inhibited by clonidine.

Bousquet & Guertzenstein (1973) reported a rise of blood pressure in the cat due to the action of clonidine on the hypothalamus and localized the hypotensive action of this drug to the medulla oblongata. The dose employed by these authors was quite high (10-100 μg i.c.v.) compared to the doses (1-2 μg) used in our experiments. Our results agree with these authors as far as the action of clonidine on the medulla is concerned. We have made no attempt to localize the compound to the hypothalamus alone. It is likely that the inhibition of hypothalamic responses following intracerebroventricular or intravertebral arterial administration of clonidine in our experiments is due to an action at the medullary level since similar inhibition is also observed following topical application of the drug to the floor of the IVth ventricle (Figure 3). However, Boudier & Van Rossum (1972) reported hypotension and bradycardia following local injection of clonidine into the hypothalamus of the rat. This variation in result may be due to a species difference.

An analysis of the results obtained in the present study clearly indicates that the inhibition of excitability of vasomotor neurones can be obtained even with doses of clonidine which do not produce hypotension in our experiments. Similar findings have been reported by us with 6-hydroxydopamine (Gupta *et al.*, 1972) and α-methylnoradrenaline (Singh, Srimal & Dhawan, 1973). We have also found (Bapat, Dhawan & Srimal, 1969) that (−)-N-(1-phenylethyl)-guanidine could facilitate centrally evoked pressor responses in a dose which had no effect on the blood pressure. Higher doses produced a rise in blood pressure as well. It appears that alteration in the excitability of vasomotor neurones is a more sensitive index of drug action on the central cardiovascular loci than changes in the blood pressure level.

Starke & Montel (1973) reported a decrease in

the output of noradrenaline due to electrical stimulation of cerebral neurones following clonidine administration. They attributed this decrease in output to stimulation of the α-adrenoceptors by clonidine. It is likely that there is an adrenergic link in the pathway which mediates the pressor responses obtained due to stimulation of the vasomotor loci and the decrease in the output of the transmitter is due either to stimulation of the α-receptors (clonidine) or to blockade of the adrenergic transmission (6-hydroxydopamine; Gupta *et al.*, 1972) which results in the decrease of the vasomotor responses and may ultimately, in higher doses, produce hypotension.

There has been a divergence of opinion regarding the role of central effects of clonidine in the hypotension observed following therapeutic doses of this drug. Because Katic, Lavery & Lowe (1972) found neither hypotension nor any evidence of central inhibitory action of clonidine (20 μg/kg) in dogs on chronic oral administration, they suggested that the central effect of clonidine was unimportant for producing hypotension in therapeutic doses. However, since the dose was without any effect, it can hardly be used as evidence against a central action. On the other hand Sherman *et al.* (1968) consider the central action of clonidine to be quite significant in producing hypotension. In the present series of experiments, effects have been obtained following central administration of low doses of clonidine which have no effect when injected peripherally. It is reasonable to assume that such small amounts could reach the central nervous system following administration of clonidine in therapeutic doses. The inhibition of central vasomotor loci is, therefore, likely to contribute significantly to the hypotension and bradycardia following systemic administration of clonidine.

This is Communication No. 1848 from the Central Drug Research Institute, Lucknow, India. D.V. was supported by the University Grants Commission, New Delhi and M.B.J. by the Indian Council of Medical Research, New Delhi, India. Clonidine was kindly donated by Boehringer, Ingelheim, Germany.

References

BAPAT, S.K., DHAWAN, B.N. & SRIMAL, R.C. (1969). Analysis of the central vasomotor effects of (−)-N-(1-phenylethyl) guanidine: A new adrenergic neurone blocking agent. *Canad. J. Physiol. Pharmac.*, **47**, 725-729.

BOUDIER, H.A.J.S. & VAN ROSSUM, J.M. (1972). Clonidine induced cardiovascular effects after stereotaxic application in the hypothalamus of rats. *J. Pharm. Pharmac.*, **24**, 410-411.

BOUSQUET, P. & GUERTZENSTEIN, P.G. (1973). Localization of the central cardiovascular action of clonidine. *Br. J. Pharmac.*, **49**, 573-579.

FELDBERG, W. & SHERWOOD, S.L. (1953). A permanent cannula for intraventricular injections in cat. *J. Physiol., Lond.*, **120**, 3-4.

GUPTA, P.P., SRIMAL, R.C. & DHAWAN, B.N. (1972). Central cardiovascular effects of 6-hydroxydopamine. *Eur. J. Pharmac.*, **20**, 215-223.

HUKUHARA, T., JR., OTSUKA, Y., TADEKA, R. & SAKAI, F. (1968). Die zentralen Wirkungen des 2-(2,6-Dichlorophenylamino)-2-imidazolin hydrochloride. *Arzneimittel-Forsch*, **18**, 1147-1153.

JASPER, H.H. & AJMONE-MARSON, C. (1954). *A Stereotaxic Atlas of the Diencephalon of the Cat.* The National Research Council of Canada.

KATIC, F., LAVERY, H. & LOWE, R.D. (1972). The central action of clonidine and its antagonism. *Br. J. Pharmac.*, **44**, 779-787.

MALING, H.M., CHO, A.K., HORAKOVA, Z. & WILLIAMS, M.A. (1969). The pharmacologic effects of ST-155 (Catapres) and related imidazolines in the rat. *Pharmacology*, **2**, 337-351.

SCHMITT, H. & SCHMITT, H. (1969). Localization of the hypotensive effect of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (St 155, Catapresan). *Eur. J. Pharmac.*, **6**, 8-12.

SHAW, J., HUNYOR, S.N. & KORNER, P.I. (1971). Sites of central nervous system action of clonidine on reflex autonomic function in the unanaesthetized rabbit. *Eur. J. Pharmac.*, **15**, 66-78.

SHERMAN, G.F., GRECA, G.I., WOODS, R.J. & BUCKLEY, J.R. (1968). Evidence for a central hypotensive mechanism for 2-(2,6-dichlorophenylamino)-2-imidazoline (Catapresan, St 155). *Eur. J. Pharmac.*, **2**, 326-328.

SINGH, G.B., SRIMAL, R.C. & DHAWAN, B.N. (1973). Inhibition by alphamethylnoradrenaline of central vasomotor loci in cat. *Pharmac. Res. Comm.*, **5**, 329-336.

SMITH, O.A. (1974). Reflex and central mechanisms involved in the control of the heart and circulation. *A. Rev. Physiol.*, **36**, 93-123.

STARKE, K. & MONTEL, H. (1973). Involvement of α-receptors in clonidine-induced inhibition of transmitter release from central monoamine neurones. *Neuropharmacology*, **12**, 1073-1080.

WHITE, C.A. & BORISON, H.L. (1955). Cardiovascular and respiratory responses to elevation of cerebrospinal fluid pressure in the cat. *Ann. N.Y. Acad. Sci.*, **62**, 33-67.

(Received September 23, 1974.
Revised January 6, 1975)