Br. J. Pharmac. (1979), **67**, 133–141

α_2-ADRENOCEPTORS MEDIATE CLONIDINE-INDUCED SEDATION IN THE RAT

G.M. DREW, ALMA J. GOWER & A.S. MARRIOTT

Department of Pharmacology, Glaxo-Allenburys Research (Ware) Ltd., Ware, Hertfordshire SG12 0DJ

1 The central α-adrenoceptors responsible for mediating clonidine-induced sedation in rats have been characterized according to their sensitivity to α-adrenoceptor agonists and antagonists.

2 Clonidine, injected intraperitoneally or intracerebroventricularly, caused dose-dependent sedation, both in terms of a reduction in the time that rats could remain on an accelerating rotarod and in terms of overt sedation assessed visually. Following intracerebroventricular injection, xylazine, naphazoline and methoxamine, but not phenylephrine, produced similar effects.

3 The sedation caused by intraperitoneal injection of clonidine was antagonized by intracerebroventricularly injected phentolamine, yohimbine, piperoxan and tolazoline but not by labetalol, thymoxamine or prazosin.

4 The relative potencies of the agonists in causing sedation and of the antagonists in inhibiting the sedative effect of clonidine clearly demonstrated that the central α-adrenoceptors mediating clonidine-induced sedation are the same as the peripheral presynaptic α_2-adrenoceptors.

5 All the α-adrenoceptor agonists caused hypothermia after intracerebroventricular injection, but their order of potency was different from that in producing sedation. The hypothermic effect of intraperitoneally injected clonidine was little affected by any of the antagonists administered intracerebroventricularly. No conclusions could be drawn concerning the type of receptor responsible for mediating hypothermia.

Introduction

One of the most common side-effects of the treatment of hypertension with clonidine is sedation which, like the hypotensive action, results from the stimulation of central α-adrenoceptors (Brüner & Klein, 1968; van Zwieten, 1975). However, the central α-adrenoceptors mediating the sedative effect of clonidine are not identical with those in peripheral vascular smooth muscle (Delbarre & Schmitt, 1971; 1973). It is now well established that peripheral α-adrenoceptors fall into two different types, which have been tentatively subclassified as α_1 and α_2 (Langer, 1974; 1977). The α_2-adrenoceptors have been identified on the terminals of the sympathetic nerves supplying the rabbit and rat heart (Starke, 1972; Drew, 1976; Cavero, Lefèvre & Roach, 1977) and rabbit pulmonary artery (Starke, Endo & Taube, 1975; Borowski, Starke, Ehrl & Endo, 1977), the motor nerves to the rat vas deferens (Drew, 1977; Doxey, Smith & Walker, 1977) and the cholinergic nerves of the guinea-pig myenteric plexus (Wikberg, 1978; Drew, 1978). They differ from the α_1-adrenoceptors located postsynaptically, in these and other tissues, in being much less sensitive to the agonists phenylephrine and methoxamine, and to the antagonists thymoxamine, labetalol and prazosin.

In the light of these findings, we have re-investigated, in detail, the sensitivity towards α-agonists and antagonists of the central α-adrenoceptors that mediate clonidine-induced sedation, in order to determine whether they resemble α_1 or α_2-adrenoceptors.

A preliminary account of these findings has been presented to the British Pharmacological Society (Drew, Gower & Marriott, 1977).

Methods

Experimentally naïve, male hooded rats (AH/H strain) were used. After transfer from the breeding unit the rats were housed 10 per cage in cages 45 × 33 × 16 cm high for 6 days before use. Apart from once daily replenishment of food (41B cube diet) and water, the rats were left undisturbed during this period. At the onset of experimentation the rats weighed 40 to 70 g. Housing and laboratory temperatures were maintained at 20 to 21°C.

Measurement of sedation and core temperature

Drug-induced sedation was first measured in terms

0007-1188/79/090133-09 $01.00

of a reduction in the time that rats were able to remain on an accelerating rotarod. Whilst this procedure provided an objective measure of sedation, it was considered unlikely that it would be selective for this type of drug action. Therefore, sedation was also measured in terms of general behavioural depression (overt sedation) which was assessed by direct observation using a behavioural check-list. It was further considered that sedation could be secondary to a hypothermic action of the drugs and so their effects on core temperature were also recorded. In case the sedation was secondary to a fall in blood pressure, the effects of hydralazine, a potent, peripherally-acting hypotensive agent, were also determined in both sedation tests.

The rotarod was based on that described by Jones & Roberts (1968). It was 3.2 cm in diameter and accelerated linearly from 0 rev/min at time 0 to a maximum of 50 rev/min at 5 min. The rod was compartmented so that 6 rats could be tested simultaneously. The rats were removed from their home cages, injected with drug or vehicle and returned to their cages for the duration of the dose-test interval. The rats were then placed on the rod facing the direction of rotation. When the rotarod was set in motion, a digital timer for each compartment started and was stopped automatically when the rat fell from the rod. In all rotarod experiments dose-groups of 6 to 8 rats were used.

Visual assessment of overt sedation and core temperature measurement were carried out separately from the rotarod experiments, with groups of 5 rats per dose. The rats were removed from their home cages, injected with drug or vehicle and returned to their cages until the time of their testing. Each rat was then removed from the cage, core temperature recorded and overt sedation assessed. Core temperature was measured by insertion of a thermistor probe (L. Light Labs. Ltd.) into the oesophagus for a few seconds until a constant temperature reading was obtained. Overt sedation was assessed by transferring the rat to a 50 × 50 cm enclosure on the laboratory bench and observing for gross differences from control rats. The following indices were used and were scored on a 0 to 4 basis according to severity: ptosis, lowered body posture, slow gait, depressed response to light pressure between finger and thumb placed on either side of body, depressed response to a rod passed to and fro across the visual field, passivity (assessed by whether or not the rat struggled when picked up gently by the dorsal fold of loose skin of neck, held gently on its back, held suspended by a fore or hind limb) and impaired righting reflex (assessed by the number of times the rat failed to land on all 4 feet when dropped 4 times from an inverted position on to a tray of sawdust). Scores for all 7 indices were summed for each rat and mean values calculated for each dose-group, the maximum possible score being 28.

Intracerebroventricular injections

The rats were lightly anaesthetized with 2% fluothane in a nitrous oxide and oxygen (4:3 v/v) mixture. Drugs or saline (0.9% w/v NaCl solution) were injected directly through the skull into the right lateral ventricle with the aid of a steel template, as described in detail by Popick (1976). The rats appeared fully recovered from the anaesthetic within 2 min of the injection.

Experimental protocol

In preliminary studies it was established that the peak sedative effect of clonidine occurred 20 min after intraperitoneal injection and remained undiminished for approximately a further 40 min. The peak hypotensive effect of hydralazine in rats occurs 30 to 60 min after intraperitoneal injection (Stanton, 1971). Thus, when these two drugs were compared their effects were measured 30 min after administration.

The relative potencies of the α-adrenoceptor agonists in causing sedation were determined after intracerebroventricular injection; this route was chosen to avoid differences in the abilities of the drugs to penetrate the blood-brain barrier. By this route the peak sedative effects of clonidine occurred approximately 15 min after injection, and so the effects of the other agonists were determined using the same interval. Each agonist was investigated in a separate experiment.

In further studies in which the interactions between clonidine and the α-adrenoceptor antagonists were examined, clonidine (0.1 mg/kg) or saline (10 ml/kg) was injected intraperitoneally 10 min after intracerebroventricular injection of the antagonist or vehicle and testing was carried out after a further 20 min. Each antagonist was investigated in a separate series of experiments.

In all experiments rats from the various drug and control treatment groups were dosed and tested in a random sequence and coded so that the observer did not know the nature of the drug treatment.

Statistics

Comparisons between groups in the rotarod and overt sedation experiments were made by the non-parametric Mann Whitney U test (Siegel, 1956). Comparisons of core temperatures between groups were made with Student's t test. Throughout all experiments, for ease of comparison, means ± s.e. are presented as the index of response. In the rotarod test ED_{50} values were the doses of drugs producing a 50%

reduction in the time that rats could remain on the rotarod compared with vehicle-treated controls. These were determined from a linear curve fitted to the log-effect data by the method of least squares. The 95% confidence limits were estimated according to Fieller's Theorem (Finney, 1971). Slopes of the log dose effect regression curves were calculated as the change in performance time per doubling of dose.

Drugs

The drugs used were: clonidine hydrochloride (Boehringer Ingelheim), hydralazine (Ciba), labetalol hydrochloride (AH 5158; Glaxo-Allenburys Research (Ware) Ltd.), (±)-methoxamine hydrochloride (Burroughs Wellcome), naphazoline nitrate (Ciba), oxymetazoline hydrochloride (Merck), phentolamine mesylate (Ciba), (−)-phenylephrine hydrochloride (Koch-Light), piperoxan hydrochloride (May and Baker), prazosin hydrochloride (Pfizer), thymoxamine hydrochloride (Warner), tolazoline hydrochloride (Ciba), xylazine hydrochloride (BAY-1470; Bayer AG) and yohimbine hydrochloride (Sigma). All drugs except prazosin and yohimbine were dissolved in saline immediately before use. Yohimbine was dissolved in distilled water. Prazosin was dissolved in a 1% solution of 1,3-dioxalan; preliminary experiments showed that this solvent alone produced no behavioural effects or changes in core temperature. The drugs were administered either intraperitoneally in a volume of 10 ml/kg or intracerebroventricularly in a volume of 20 µl per rat. All drug doses refer to the free base. Non-drug-treated control animals received an equivalent volume of vehicle except in yohimbine experiments when control rats received saline.

Results

Sedative and hypothermic actions of intraperitoneally administered clonidine and hydralazine

The effects of intraperitoneal injections of clonidine, 0.05 to 0.2 mg/kg, and hydralazine, 0.5 to 2 mg/kg, in causing sedation and on core temperature are shown in Figure 1. Clonidine caused sedation in both tests and reduced core temperature. These effects were dose-related. In contrast, hydralazine caused no significant sedation, although hypothermia did occur at the highest dose level. In our experience hydralazine reduces blood pressure in DOCA hypertensive rats to a similar or greater extent than clonidine at the doses used in the present experiments.

Sedative and hypothermic actions of α-adrenoceptor agonists injected intracerebroventricularly

The effects of α-adrenoceptor agonists, injected intracerebroventricularly, in causing sedation and on core temperature are shown in Figure 2. With the exception of phenylephrine, all of the drugs impaired rotarod performance and produced overt sedation and hypothermia. In most cases these effects were dose-related. Unlike the other drugs tested, phenylephrine, although causing hypothermia, did not cause significant sedation over the range of doses shown. Higher doses of this drug proved lethal. ED_{50} values for the α-adrenoceptor agonists in causing deficits in rotarod performance are shown in Table 1. Also

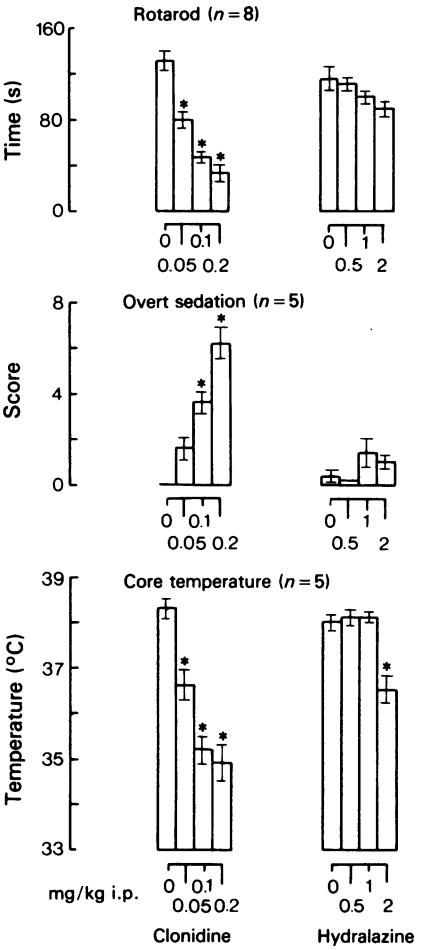

Figure 1 Effects of clonidine and hydralazine, following intraperitoneal injection, in causing sedation and changes in core temperature. Sedation was measured both in terms of a decrease in the length of time that rats could remain on an accelerating rotarod and in terms of overt sedation. Values plotted are means with vertical bars indicating s.e. means; *n* values indicate group sizes. *Significantly different from control rats injected intraperitoneally with saline ($P < 0.05$, 2-tailed).

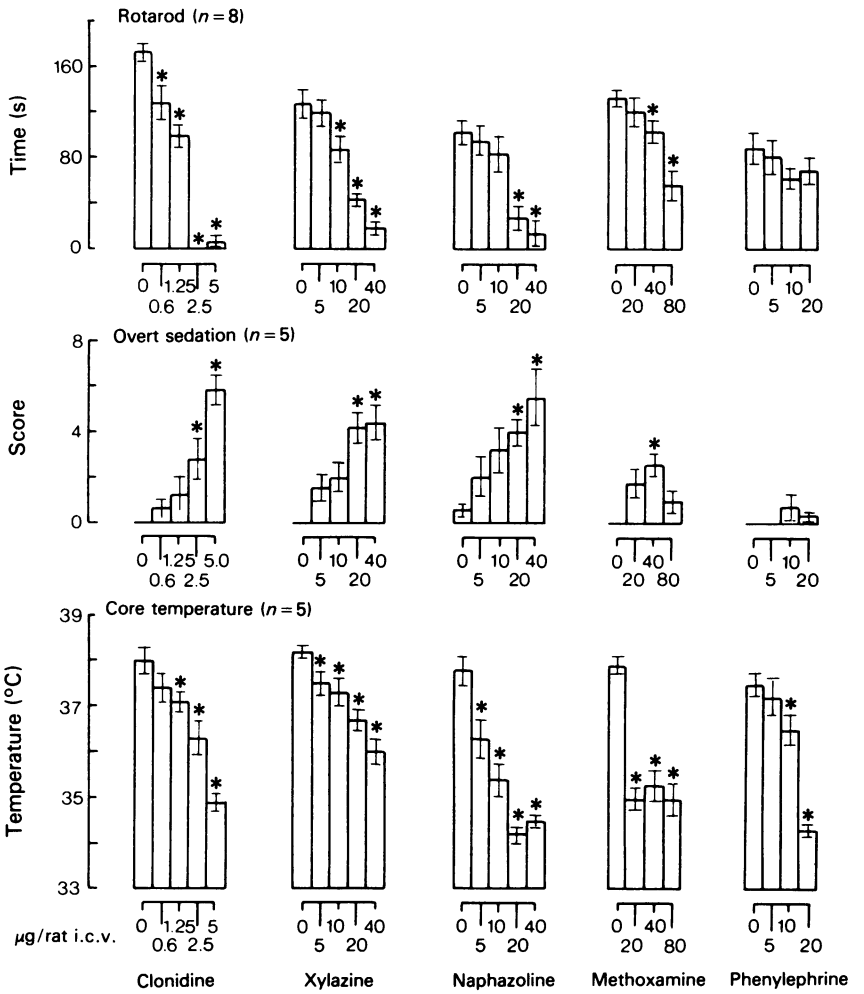

Figure 2 Effects of α-adrenoceptor agonists, following intracerebro-ventricular injection, in causing sedation and changes in core temperature. Sedation was measured both in terms of a decrease in the length of time that rats could remain on an accelerating rotarod and in terms of overt sedation. Values plotted are means with vertical bars indicating s.e. means; *n* values indicate group sizes. *Significantly different from control rats injected intracerebroventricularly with vehicle ($P < 0.05$, 2-tailed).

Table 1 ED_{50} values and slopes* for α-adrenoceptor agonists in causing deficits in rotarod performance

Agonist	*ED_{50} (95% conf. limits) μg/rat i.c.v.*	*Slope*
Clonidine	1.2 (0.9–1.4)	−32.0
Xylazine	15.1 (10.7–24.5)	−32.7
Naphazoline	15.4 (10.6–22.9)	−30.2
Methoxamine	67.8 (51.6–94.7)	−34.8
Phenylephrine	Inactive up to 20	—

* ED_{50} values and slopes are defined in the statistics section of the Methods.

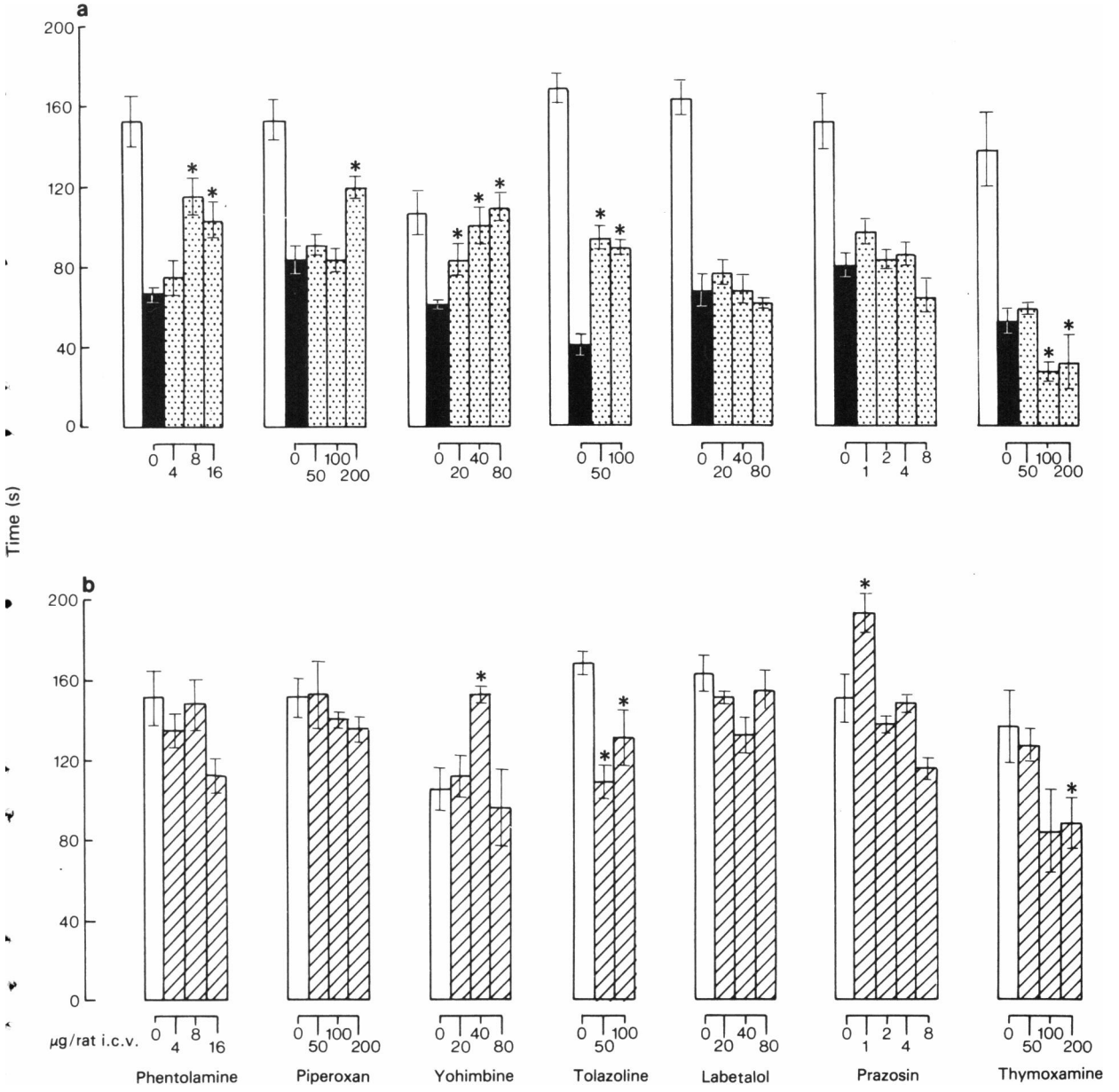

Figure 3 (a) Effects of α-adrenoceptor antagonists on clonidine-induced decreases in the time that rats could remain on an accelerating rotarod. The antagonists were injected intracerebroventricularly 10 min before an intraperitoneal injection of clonidine, 0.1 mg/kg (stippled columns). Control rats were injected intracerebroventricularly with vehicle and intraperitoneally with either clonidine (solid columns) or saline (open columns). (b) Effects of α-adrenoceptor antagonists alone on the time that rats could remain on the rotarod. Rats were injected intracerebroventricularly with α-antagonist (hatched columns) or with vehicle (open columns) 10 min before an intraperitoneal injection of saline. All rats were tested on the rotarod 20 min after intraperitoneal injection. Values plotted are means from groups of 6 rats. Vertical bars indicate s.e. means. $*P < 0.05$ (2-tailed) vs vehicle-clonidine control (a) or vs vehicle-saline control (b).

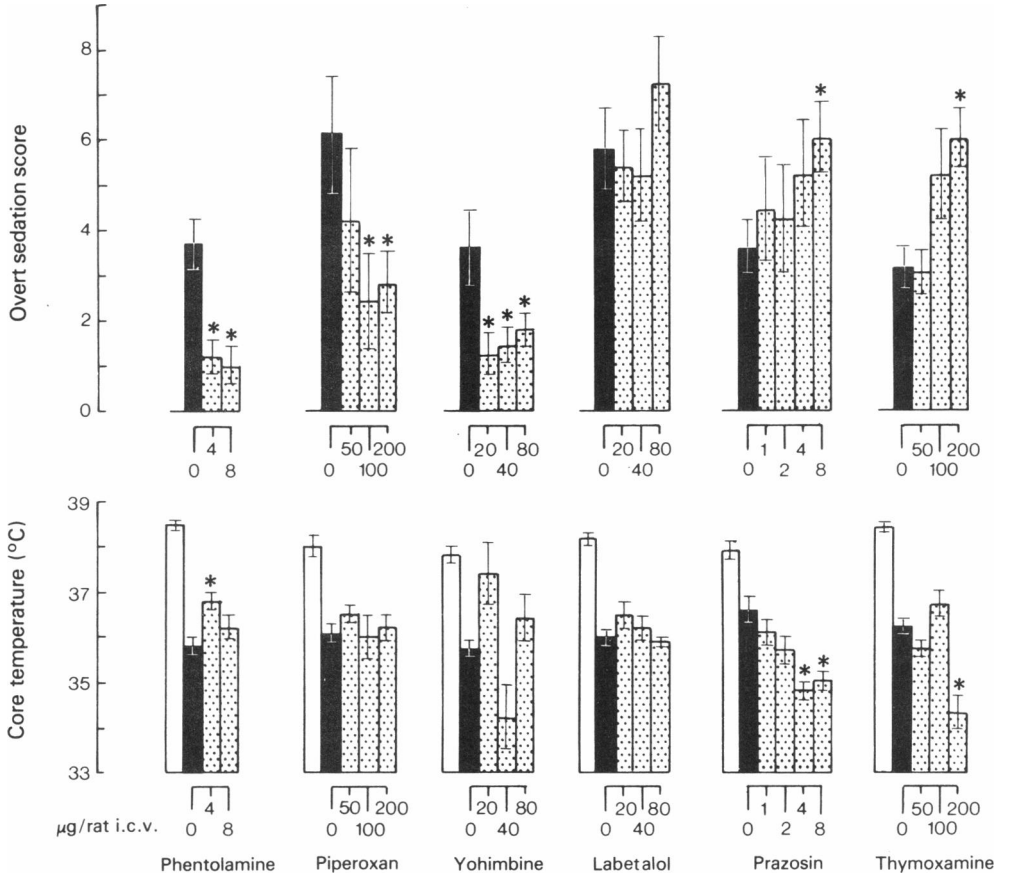

Figure 4 Effects of α-adrenoceptor antagonists on overt sedation and hypothermia induced by clonidine in rats. The antagonists were injected intracerebroventricularly 10 min before an intraperitoneal injection of clonidine, 0.1 mg/kg (stippled columns). Control rats were injected intracerebroventricularly with vehicle and intraperitoneally with either clonidine (solid columns) or saline (open columns). All rats were tested for sedation and changes in core temperature 20 min after intraperitoneal injection. Values plotted are means from groups of 5 rats. Vertical bars indicate s.e. means. Open columns are absent from overt sedation histograms because no sedation occurred in the vehicle-saline controls. *Significantly different from vehicle-clonidine controls ($P < 0.05$, 2-tailed).

shown are the slopes of the log dose effect regression curves. The effects of oxymetazoline, 5 to 40 μg per rat intracerebroventricularly, were also examined. It produced sedation at 5 but not at 15 min after injection. At 10 and 15 min after injection oxymetazoline caused exophthalmus and piloerection. This suggested that the drug was being rapidly removed from the brain. In view of these findings no further experiments were carried out with oxymetazoline.

Effects of α-adrenoceptor antagonists on clonidine-induced impairment of rotarod performance

The effects of α-adrenoceptor antagonists, injected intracerebroventricularly 10 min before intraperitoneal clonidine, on clonidine-induced deficits in rotarod performance are shown in Figure 3a. It can be seen that phentolamine, piperoxan, yohimbine and tolazoline inhibited the sedative action of clonidine as measured by rotarod performance. In contrast, labetalol, prazosin and thymoxamine did not inhibit the effects of clonidine; thymoxamine, in fact, increased the effect of clonidine.

The effects of the antagonists, given alone, on rotarod performance are shown in Figure 3b. Phentolamine, piperoxan and labetalol were without effect on rotarod performance. Yohimbine and prazosin generally had little effect although one dose of each drug increased performance. Tolazoline and thymoxamine both reduced rotarod performance. Thus, it is

clear that the ability of the antagonists to inhibit clonidine-induced changes in rotarod performance is independent of their own effects in this test.

Effects of α-adrenoceptor antagonists on overt sedation and hypothermia induced by clonidine

The effects of α-adrenoceptor antagonists, injected intracerebroventricularly 10 min before intraperitoneal clonidine, on overt sedation and hypothermia induced by clonidine are shown in Figure 4. The effects of tolazoline were not investigated in these experiments. Phentolamine, piperoxan and yohimbine antagonized clonidine-induced sedation. Labetalol did not antagonize clonidine-induced sedation; thymoxamine and prazosin increased it. These results were therefore closely similar to the results obtained in the rotarod experiments. When the α-antagonists were tested alone only thymoxamine, 100 and 200 μg per rat, caused significant overt sedation and none caused behavioural excitation.

Unlike the clonidine-induced sedation, hypothermia was not antagonized by piperoxan or yohimbine and was not consistently antagonized by phentolamine (Figure 4). Clonidine-induced hypothermia was increased by prazosin and thymoxamine but was unaffected by labetalol. No significant changes in core temperature occurred following yohimbine, labetalol or prazosin alone; phentolamine, 4 and 8 μg/rat, piperoxan 200 μg/rat and thymoxamine, 200 μg/rat, caused significant hypothermia.

Discussion

The present results confirm earlier reports that clonidine causes sedation in animals (Laverty, 1970; Holman, Shillito & Vogt, 1971; Delbarre & Schmitt, 1971; 1973). This effect was seen when clonidine was administered intraperitoneally or intracerebroventricularly. The results also confirm that clonidine reduces body temperature (Laverty & Taylor, 1969; Tsoucaris-Kupfer & Schmidt, 1972). The sedative effect of clonidine is unlikely to be a result of the fall in body temperature, because phenylephrine, although causing hypothermia, did not cause sedation and other drugs which antagonized the sedative effects of clonidine, did not antagonize clonidine-induced hypothermia. Neither is the sedation likely to be linked to a fall in blood pressure because hydralazine did not cause sedation.

α-Agonists other than clonidine also caused sedation; the rank order of the agonists was clonidine > xylazine ≡ naphazoline > methoxamine > phenylephrine. The similarity in the log dose effect regression curves suggests that all the agonists exerted their effects via a common mechanism. Their relative potencies, assigning clonidine the value of unity, were 1:12.6:12.8:56.5: > 16 respectively. This is almost identical with the relative potencies previously reported with the same agonists at the presynaptic α_2-adrenoceptors in the rat heart (Drew, 1976), which were 1:20.5:12.7:141.6: > 13 respectively. Thus, the present results strongly suggest that α_2-adrenoceptors mediate the sedative effects of clonidine, a view that is supported by the results obtained with the antagonists. Only those antagonists previously found to antagonize the effects of clonidine at peripheral α_2-adrenoceptors (Drew, 1976; 1977; 1978; Cavero *et al.*, 1977; Doxey & Easingwood, 1978) antagonized the sedative effects of clonidine. Very similar results have been reported recently from experiments carried out with chicks (Cavero & Roach, 1978; Roach, Lefèvre-Borg & Cavero, 1978).

Although the α_2-adrenoceptors so far identified in the periphery are all located presynaptically it does not necessarily follow that the central α_2-adrenoceptors mediating the clonidine-induced sedation are also located presynaptically. However, there is some evidence that they might be: it has been shown (Zebrowska-Lupina, Przegalinski, Sloniec & Kleinrok, 1977) that clonidine reduces locomotor activity in normal rats but greatly increases it in rats in which central catecholamine and indoleamine stores have been depleted. The increased locomotor activity was believed to be mediated via postsynaptic α-adrenoceptors. The sensitivity of these receptors to antagonists, allowing for differences in pretreatment times and access to the central nervous system, suggests that they resemble the postsynaptic α_1-adrenoceptors found in rabbit pulmonary artery (Borowski *et al.*, 1977) and rat vascular smooth muscle (Drew, 1976). Thus it seems likely that the α_2-adrenoceptors that mediate clonidine-induced sedation in normal rats are located presynaptically.

If the α_2-adrenoceptors identified in the present study are located presynaptically the slight, but significant, effect of yohimbine alone in facilitating rotarod performance could be explained on the basis of its greater potency in blocking the α_2 than the α_1-adrenoceptors (Borowski *et al.*, 1971; Starke, Borowski & Endo, 1975; Doxey *et al.*, 1977). This would lead to a greater release of transmitter and thus to effects similar to those produced by clonidine at the postsynaptic α-adrenoceptors. It is unlikely that the deficit in rotarod performance produced by the highest doses of tolazoline and thymoxamine are attributable to selective postsynaptic blockade because labetalol and prazosin, which are also selective antagonists at α_1-adrenoceptors do not reduce rotarod performance. Thus, we have no explanation for these effects of tolazoline and thymoxamine, or for the small increase in rotarod performance seen with one dose of prazosin.

There is also evidence that the α_2-receptors might be located postsynaptically. The cell bodies of the locus coeruleus are believed to receive an input from *adrenaline* containing nerves; activity in the coerulocortical pathway maintains wakefulness (Fuxe, Bolme, Hockfelt & Goldstein, 1975). Cedarbaum & Aghajanian (1977) have shown that microiontophoretic application of α-agonists into this region inhibits the spontaneous firing of locus coeruleus neurones by stimulating α_2-adrenoceptors, which the authors concluded were located on, or near, the cell bodies of the locus coeruleus neurones. An alternative interpretation of these findings is that the α-agonists inhibited cell discharge not by an action on the cell bodies themselves, but by stimulating α_2-adrenoceptors on the terminals of the nerves innervating the cell bodies of the locus coeruleus thereby reducing spontaneous transmitter release.

The results of the present experiments confirm those of Tsoucaris-Kupfer & Schmitt (1972) who demonstrated that intraperitoneal injection of clonidine caused a dose-related reduction in body temperature. In contrast to these authors, we found that clonidine also reduced body temperature after intracerebroventricular injection, as did all the other agonists. However, the order of potency of the agonists in causing hypothermia was different from that in causing sedation. It also differs from orders of potency obtained at peripheral postsynaptic α_1-adrenoceptors and presynaptic α_2-adrenoceptors in pithed rats (Drew, 1976). In addition some, but not all of the *antagonists* reduced body temperature after intracerebroventricular injection, but this effect did not appear to be related to the antagonists' ability to block α_1- or α_2-adrenoceptors. Finally, the hypothermic effect of intraperitoneally injected clonidine was little affected by any of the antagonists even in doses that antagonized its sedative effects. Thus we are unable to draw any conclusions concerning the receptors that mediate hypothermia, but they are clearly different from those mediating sedation.

An interesting point that arises from the experiments described here is that it may prove difficult to separate the sedative and hypotensive properties of clonidine-like drugs because the clonidine-induced fall in blood pressure also seems to be mediated primarily via central α_2-adrenoceptors (Hersom, Finch & Metcalf, 1978). We have obtained results (unpublished) with guanfacine (BS 100-141) that support this view. Guanfacine is a centrally acting anti-hypertensive agent that is about 10 times less potent than clonidine, but which is reputed to cause little or no sedation in animals (Scholtysik, Lauener, Eichenberger, Burki, Salzmann, Muller-Schweinitzer & Waite, 1975; Kleinlogel, Scholtysik & Sayers, 1975; Saameli, Scholtysik & Waite, 1977). However, we have found that guanfacine is 20-25 times less potent than clonidine in causing sedation and hypothermia following intraperitoneal injection. These findings confirm recent clinical experience (Jäättelä, 1976a, b) and suggest that the test procedures used in these experiments may predict the sedative effects of such drugs in man.

The authors gratefully acknowledge Mr S.P. Close, Miss S.M. Leigh and Miss V.A. Stokes for skilful technical assistance, and the various companies for their generous gifts of clonidine, prazosin, thymoxamine and xylazine.

References

BOROWSKI, E., STARKE, K., EHRL, H. & ENDO, T. (1977). A comparison of pre- and post synaptic effects of α-adrenolytic drugs in the pulmonary artery of the rabbit. *Neuroscience*, **2**, 285–296.

BRÜNER, H. & KLEIN, K.E. (1968). Experimentelle Untersuchungen uber die Veranderungen der psychomotorischen Leistungsbereitschaft unter dem Einfluss eines Antihypertonikums. In *Hochdrucktherapie*. ed Heilmeyer L., Holtmeier H-J. & Pfeiffer E.F. pp. 180–184. Stuttgart: G. Thieme.

CAVERO, I., LEFÈVRE, F. & ROACH, A.G. (1977). Further studies on the cardio-vascular effects of prazosin. *Fedn Proc.*, **36**, 955.

CAVERO, I. & ROACH, A.G. (1978). The effects of prazosin on the clonidine induced hypotension and bradycardia in rats and sedation in chicks. *Br. J. Pharmac.*, **62**, 468P–469P.

CEDARBAUM, J.M. & AGHAJANIAN, G.K. (1977). Catecholamine receptors on locus coeruleus neurons: pharmacological characterization. *Eur. J. Pharmac.*, **44**, 375–385.

DELBARRE, B. & SCHMITT, H. (1971). Sedative effects of α-sympathomimetic drugs and their antagonism by adrenergic and cholinergic blocking drugs. *Eur. J. Pharmac.*, **13**, 356–363.

DELBARRE, B. & SCHMITT, H. (1973). A further attempt to characterize sedative receptors activated by clonidine in chickens and mice. *Eur. J. Pharmac.*, **22**, 355–359.

DOXEY, J.C. & EASINGWOOD, R.E. (1978). Profiles of α-adrenoceptor antagonists in the pithed rat. *Br. J. Pharmac.*, **63**, 401–402.

DOXEY, J.C., SMITH, C.F.C. & WALKER, J.M. (1977). Selectivity of blocking agents for pre- and postsynaptic α-adrenoceptors. *Br. J. Pharmac.*, **60**, 91–96.

DREW, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists at pre- and postsynaptically located α-adrenoceptors. *Eur. J. Pharmac.*, **36**, 313–320.

DREW, G.M. (1977). Pharmacological characterisation of the presynaptic α-adrenoceptor in the rat vas deferens. *Eur. J. Pharmac.*, **42**, 123–130.

DREW, G.M. (1978). Pharmacological characterisation of the presynaptic α-adrenoceptors regulating cholinergic

activity in the guinea-pig ileum *Br. J. Pharmac.*, **64**, 293–300.

DREW, G.M., GOWER, A.J. & MARRIOTT, A.S. (1977). Pharmacological characterisation of α-adrenoceptors which mediate clonidine-induced sedation. *Br. J. Pharmac.*, **61**, 468p.

FINNEY, D.J. (1971). *Statistical Method in Biological Assay*. London: Griffin.

FUXE, K., BOLME, P., HOKFELT, T. & GOLDSTEIN, M. (1975). Central adrenaline receptors and blood pressure regulation: evidence for an adrenergic vaso-depressor system mediating the actions of clonidine. In *Workshops of the Symposium on Recent Advances in Hypertension*, vol. 3, ed. Milliez, P. & Safar, M. pp. 72–80. Monaco: Laboratories Boehringer-Ingelheim.

HERSOM, A.S., FINCH, L. & METCALF, G. (1978). Interactions between clonidine and α-adrenoceptor antagonists: *Proc. 7th International Congress of Pharmacology*, Abstr. 2713, (in press).

HOLMAN, R.B., SHILLITO E.E. & VOGT, M. (1971). Sleep produced by clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride). *Br. J. Pharmac.*, **43**, 685–695.

JÄÄTTELÄ, A. (1976a). Clinical efficacy of BS100-141 in essential hypertension. *Eur. J. clin. Pharmac.*, **10**, 69–72.

JÄÄTTELÄ, A. (1976b). Comparison of BS100-141 and clonidine as antihypertensive agents. *Eur. J. clin. Pharmac.*, **10**, 73–76.

JONES, B.J. & ROBERTS, D.J. (1968). The quantitative measurement of motor inco-ordination in naïve mice using an accelerating rotarod. *J. Pharm. Pharmac.*, **20**, 302–304.

KLEINLOGEL, H., SCHOLTYSIK, G. & SAYERS, A.C. (1975). Effects of clonidine and BS100-141 on the EEG sleep pattern in rats. *Eur. J. Pharmac.*, **33**, 159–163.

LANGER, S.Z. (1974). Presynaptic regulation of catecholamine release. *Biochem. Pharmac.*, **23**, 1793–1800.

LANGER, S.Z. (1977). Sixth Gaddum Memorial Lecture. Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.*, **60**, 481–497.

LAVERTY, R. (1970). A comparison of the behavioural effects of some hypotensive imidazoline derivatives in rats. *Eur. J. Pharmac.*, **9**, 163–169.

LAVERTY, R. & TAYLOR, K.M. (1969). Behavioural and biochemical effects of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (St 155) on the central nervous system. *Br. J. Pharmac.*, **35**, 253–264.

POPICK, F.R. (1976). Application of a new intraventricular injection technique in rat brain norepinephrine studies. *Life Sci., Oxford*, **18**, 197–204.

ROACH, A.G., LEFÈVRE-BORG, F. & CAVERO, I. (1978) Evidence favouring a similarity between rat cardiac presynaptic α-adrenoceptors and those mediating sleep in chicks. *Proc. 7th International Congress of Pharmacology*, Abstr. 1407. (in press).

SAAMELI, K., SCHOLTYSIK, G. & WAITE R. (1975). Pharmacology of BS100-141, a centrally acting antihypertensive drug. *Clin. exp. Pharmac. Physiol.*, **suppl. 2**, 207–212.

SCHOLTYSIK, G., LAUENER, H., EICHENBERGER, E., BURKI, H., SALZMANN, R., MÜLLER-SCHWEINITZER, E. & WAITE, R. (1975). Pharmacological actions of the antihypertensive agent N-amidino-2-(2,6-dichlorphenyl) acetamide hydrochloride (BS100-141). *Arzneim-Forsch.*, **25**, 1483–1491.

SIEGEL, S. (1956) *Nonparametric Statistics for the Behavioural Sciences*. London: McGraw-Hill.

STANTON, H.C. (1971). Experimental Hypertension. In *Methods in Pharmacology*. vol. 1. ed. Schwartz, A. pp. 125–150. New York: Appleton-Century-Croft.

STARKE, K. (1972). Alpha Sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart. *Naunyn-Schmiedebergs Arch Pharmac.*, **274**, 18–45.

STARKE, K., BOROWSKI, E. & ENDO, T. (1975). Preferential blockade of presynaptic α-adrenoceptors by yohimbine. *Eur. J. Pharmac.*, **34**, 385–388.

STARKE, K., ENDO, T. & TAUBE, H.D. (1975) Relative pre- and postsynaptic potencies of α-adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedebergs Arch. Pharmac.*, **291**, 55–78.

TSOUCARIS-KUPFER, D. & SCHMITT, H. (1972). Hypothermic effect of α-sympathomimetic agents and their antagonism by adrenergic and cholinergic blocking drugs. *Neuropharmac.*, **11**, 625–635.

WIKBERG, J. (1978). Differentiation between pre- and post junctional α-receptors in the guinea-pig ileum and rabbit aorta. *Acta physiol., scand.*, **103**, 225–239.

ZEBROWSKA-LUPINA, I., PRZEGALINSKI, E., SLONIEC, M. & KLEINROK, A. (1977). Clonidine-induced locomotor hyperactivity in rats. *Naunyn-Schmiedebergs Arch. Pharmac.*, **297**, 227–231.

VAN ZWEITEN, P.A. (1975). Antihypertensive drugs with a central action. *Prog. Pharmac.*, **1**, 1–63.

(Received December 13, 1978.
Revised February 5, 1979.)