lasting inhibitory and excitatory phases). Thus, where an excitatory response to NA was associated with, or even masked by inhibition this response is probably related to (+)-amphetamine excitation.

These results demonstrate that CPZ can antagonize the excitatory action of (+)-amphetamine on brain stem neurones. The fact that NA excitation appeared to be less susceptible to block by CPZ indicates a probable presynaptic mechanism for the CPZ/(+)-amphetamine antagonism. Such a mechanism could provide a basis for antagonism of the alerting effects of (+)-amphetamine by CPZ.

We thank Boehringer Ingelham Ltd. for the gift clonidine.

REFERENCES

BOAKES, R. J., BRADLEY, P. B. & CANDY, J. M. (1972). A neuronal basis for the alerting action of (+) – amphetamine. *Br. J. Pharmac.*, **45**, 391–403.

BRADLEY, P. B. & HANCE, A. J. (1957). The effect of chlorpromazine and methopromazine on the electrical activity of the brain in the cat. *Electroenceph. clin. Neurophysiol.* **9**, 191–215.

BRADLEY, P. B., WOLSTENCROFT, J. H., HOSLI, L. & AVANZINO, G. L. (1966). Neuronal basis for the central action of chlorpromazine. *Nature, Lond.* **212**, 1425–1427.

HELLON, R. F. (1971). The marking of electrode tip positions in nervous tissue. *J. Physiol. Lond.*, **214**, 12P.

The effects of clonidine on single cortical and medullary neurones

CAROLINE ANDERSON and T. W. STONE* (introduced by J. L. MALCOLM)

Department of Physiology, University of Aberdeen, Marischal College, Aberdeen.

Clonidine (Catapres, St. 155) has been shown to produce an initial hypertension, followed by a more prolonged hypotension after its systemic injection. The pressor effect appears to be due to stimulation of peripheral α-adrenoceptors. The hypotension appears to be due to an action on the central nervous system, and most of the available evidence suggests an interaction with noradrenaline (NA) systems in the brain. The present experiments were intended to clarify the action of clonidine in the brain by applying it directly to neurones by microiontophoresis and comparing its effects with those of NA.

Male rats anaesthetized with urethane were used in these experiments. Clonidine was ejected with a current of 80 nA from a 200 mM solution of clonidine hydrochloride (pH 5·5). Experiments were performed on 185 randomly encountered spontaneously active cells in the somatosensory cerebral cortex and 62 in the medullary reticular formation at the level of exit of the IX and X cranial nerves.

The most frequently observed effect of clonidine was a depression of neuronal firing. This effect was seen on cells which were also depressed by NA. In general, cells which would not respond to NA were unaffected by clonidine. On seven cells a potentiation of NA depression occurred after the ejection of clonidine, and on three cells there was evidence of an antagonism of NA.

On approximately 18% of all neurones tested, however, clonidine caused an increase of firing rate. This response usually had a latency of about 30 s after a 1 min application of clonidine. The response occurred even on cells which were depressed by NA.

In accordance with previous experience (Stone, 1973 ; Lake, Jordan & Phillis, 1973) few cells were encountered which were excited by NA. In these cases clonidine either did not affect the firing rate of the neurones or produced a slight excitation.

The results support the idea that clonidine can act on NA receptors in the brain, usually mimicking the depressant responses of the catecholamine. Preliminary experiments indicate that depressant responses to both substances can be specifically antagonized by bulbocapnine applied by microiontophoresis. The mechanism of clonidine's excitatory action is unclear. These responses could be secondary to an action on blood vessels as has been suggested for the excitatory effects of NA (Stone, 1971). An alternative possibility is that clonidine reduces the release of NA from nerve endings. Such a reduction of NA release has been demonstrated in the rabbit heart (Starke, Wagner & Schumann, 1972). If this effect occurs after the systemic injection of clonidine, it may explain the need for an intact NA neurone system in the production of clonidine's hypotensive effect (Dollery & Reid, 1973).

We thank Boehringer Ingelheim Ltd. for the gift of clonidine.

REFERENCES

DOLLERY, C. T. & REID, J. L. (1973). Central noradrenergic neurones and the cardiovascular actions of clonidine in the rabbit. *Br. J. Pharmac.*, **47**, 206–216.

LAKE, N., JORDAN, L. M. & PHILLIS, J. W. (1973). Mechanism of noradrenaline action in cat cerebral cortex. *Nature, New Biology*, **240**, 249–250.

STARKE, K., WAGNER, J. & SCHUMANN, H. J. (1972). Adrenergic neuron blockade by clonidine: comparison with guanethidine and local anaesthetics. *Arch. Int. Pharmacodyn.*, **195**, 219–308.

STONE, T. W. (1971). Are noradrenaline excitations artifacts? *Nature, Lond.*, **234**, 145–146.

STONE, T. W. (1973). Pharmacology of pyramidal tract cells. Noradrenaline and related substances. *Arch. Pharmacol.* In press.

The effect of α-methyl-p-tyrosine, p-chlorophenylalanine, methysergide and propranolol on CO_2-induced amnesia in rats

B. E. LEONARD and H. RIGTER*

Department of Pharmacology, Organon Scientific Development Group, Kloosterstraat 6, Oss, The Netherlands

As there are reports that CO_2-induced anaesthesia results in amnesia (Paolini, Quartermain & Miller, 1966; Taber & Banuazizi, 1966) a study has been made of the efficacy of CO_2 as an amnesic agent in rats.

The 'step-through' passive avoidance test (Ader, Weijmen & Moleman, 1972), was used. The apparatus consists of a brightly illuminated runway attached to a darkened chamber which contains a grid floor. Three pre-training trials were found to be sufficient for the rats to enter the chamber within 1–3 s of being placed on the runway.

In the first experiment, the rats were randomly divided into 4 groups of 10. At the conclusion of the fourth trial, one group (S-CO_2) received a scrambled footshock of 0·5 mA for 3 s through the grid floor of the chamber. Immediately after this, the rats were placed in a box saturated with CO_2; they were left in the box until respiratory arrest occurred and were then revived by artificial respiration.

The second group, (S) received the footshock, but was not subjected to the CO_2 treatment.

The third group (NS-CO_2) was subjected to the CO_2 treatment alone while the control group (N) was untreated. Retention of the learned response was tested 24 h later. The latency of entry into the chamber was also recorded.

When tested for retention, group S completely avoided entering the chamber. In contrast, group S-CO_2 readily entered the chamber thereby demonstrating that CO_2 induced amnesia.

In a previous study, it was found that the behavioural changes in the S and S-CO_2 groups may be associated with alterations in the metabolism of biogenic amines in the hippocampus (Leonard & Rigter, 1973). The effect of some drugs known to inhibit the synthesis of these amines, or block their receptor sites, was therefore studied on CO_2-induced amnesia.

Pretreatment with α-methyl-p-tyrosine (300 mg/kg) and propranolol (15 mg/kg) before the retrieval trial reduced the CO_2-induced amnesia; p-chlorophenylalanine (400 mg/kg) and methysergide (5 mg/kg) were less effective. (+)-Amphetamine 2 mg/kg and physostigmine (0·5 mg/kg) had no effect on the amnesia.

From these results, it appears that the amnesic effect of CO_2 is associated with changes in brain noradrenaline metabolism; 5-hydroxytryptamine may play a subsidiary role.

REFERENCES

ADER, R., WEIJNEN, J. A. W. M., & MOLEMAN, P. (1972). Rentention of a passive avoidance response as a function of the intensity and duration of electric shock. *Psychonom. Sci.* **26**, 125–128.

LEONARD, B. E. & RIGTER, H. (1973). Changes in brain monoamine metabolism associated with CO_2-induced amnesia in rats. *Br. J. Pharmac.*, **48**, 351–352*P*.

PAOLINI, R. M., QUATERMAIN, D. & MILLER, N. E. (1966). Different temporal gradients of retrograde amnesia produced by carbon dioxide anaesthesia and electroconvulsive shock. *J. Comp. Physiol. Psychol.* 62, 270–274.

TABER, R. I. & BANUAZIZI, A. (1966). Co_2-induced retrograde amnesia in a one-trial learning situation. *Psychopharmacologia* **9**, 382–391.