J. Pharm. Pharmacol., 1965, **17**, 788–793 Received May 31, 1965

Macusine B: further pharmacology

B. E. LEONARD

Macusine B reduced the induction time and potentiated the barbiturate sleeping time of mice, possibly as a consequence of the hypothermia produced by the alkaloid. There was no evidence to suggest that macusine B affects the synthesis or metabolism of noradrenaline, dopamine, 5-hydroxytryptamine or γ-aminobutyric acid in the rat brain, nor was there any evidence to suggest that it affected the binding of noradrenaline and 5-hydroxytryptamine within the brain. There is an indication that macusine B may act on central adrenergic receptors. The slight depression of the behavioural activity which occurs in rats and mice after the injection of macusine B was probably due to the hypothermia induced by the alkaloid.

IN a previous investigation, macusine B was found to act on both adrenergic and tryptamine receptors (Leonard, 1965). This alkaloid also caused convulsions when injected into rats or mice. The present study was therefore undertaken to determine whether it was possible to explain the effect of macusine B on the brain by its effect on amine levels or on adrenergic receptors within this organ.

Experimental

EFFECTS ON THE BARBITURATE SLEEPING TIME OF MICE

Amylobarbitone sodium (100 mg/kg) was injected intraperitoneally into albino mice, 18–22 g, of the same sex. This produced a sleeping time of approximately 15 min in the control group, the members of which were also injected with 0·9% saline (10 ml/kg i.p.). The experimental groups were pretreated with macusine B, 25 mg/kg i.p., for 0, 1, 2½, 4, 8, 13 and 24 hr before being injected with amylobarbitone sodium. The mice were kept at room temperature (approximately 21°) during the course of the experiment and the induction time and sleeping time recorded. The induction time was taken as the period between the injection and the loss of righting reflex, and the sleeping time as the period between the loss and the return of the righting reflex.

EFFECT ON THE BODY TEMPERATURE OF MICE UNDER ANAESTHESIA

Groups of 10 albino mice (18–26 g) were injected with amylobarbitone sodium (100 mg/kg i.p.) after their rectal temperature had been recorded using a clinical thermometer. The control group was injected with 0·9% saline (10 ml/kg i.p.) and the experimental group with macusine B (10 mg/kg i.p.). The animals were kept at room temperature (approximately 21°) during the experiment and the sleeping time determined. The rectal temperature of the mice was taken at 5–10 min intervals for 1 hr, after the injection of the alkaloid, by means of a hypothermic thermometer*. The experiment was then repeated, using other mice, at an ambient temperature of 35° by placing the mice in a well ventilated oven immediately after they had lost their righting reflex.

From the Department of Pharmacy, University of Nottingham, England.

* Brannon, London

EFFECT ON BRAIN AMINE LEVELS

Groups of 5 albino rats (90–100 g) were injected with macusine B (10 mg/kg i.p.) and decapitated ½, 1, 2, 4 and 8 hr after the injection. The brain and heart were removed rapidly and blotted free from excess blood. A piece of cerebral cortex (approximately 100 mg) was removed from the left hemisphere, weighed, ground in a glass mortar with 0·01 N hydrochloric acid and the γ-aminobutyric acid content estimated fluorimetrically by the method of Lowe, Robins & Eyerman (1958) as modified by Uchida & O'Brien (1964). The remainder of the brain and the heart were weighed and dropped into a container of liquid oxygen. The tissues were crushed in a metal anvil and extracted with 0·4 N perchloric acid. Noradrenaline and dopamine were estimated in the supernatant by the methods of Anton & Sayre (1962; 1964). A similar group of animals was used for the estimation of the effect of macusine B on 5-hydroxytryptamine (5-HT) content of the brain. The brains were frozen in liquid oxygen and crushed in an anvil before being transferred to a salt-saturated butanol-hydrochloric acid mixture. The 5-HT was then estimated, mainly by the method of Shore & Olin (1958), but owing to the finding that macusine B has a fluorescence peak in the same region of the spectrum as 5-HT when assayed in dilute hydrochloric acid, the fluorophor was developed in 3 N hydrochloric acid as described by Bogdanski, Pletscher, Brodie & Udenfriend (1956). The amine content was also estimated in the brains and hearts of a group of 10 control rats which had been injected with 0·9% saline.

EFFECT ON THE DEPLETION OF BRAIN 5-HT AND NORADRENALINE INDUCED BY RESERPINE

Two groups of 10 albino rats (90–100 g) were injected with reserpine (2 mg/kg i.p.) and decapitated 4 hr later. Group 1 was injected with reserpine alone and group 2 was injected with reserpine and macusine B (10 mg/kg i.p.). The control group was injected with the polypropylene glycol, benzyl alcohol and citric acid vehicle used to dissolve the reserpine (Leyden, Pomerantz & Bouchard, 1956). The noradrenaline and 5-HT content of the brains were determined as described above.

EFFECT ON THE ACTIVITY OF RATS

The activity was recorded using an activity cage of dimension 13 × 12 × 30 cm in which a photoelectric cell was activated every time the rat intercepted a beam of light. The photoelectric cell was connected to a post office counter and the number of counts were recorded at hourly intervals. Six albino rats, 90–100 g, were put singly into the activity cages 1 hr before the start of the experiment. At the start of the experiment 3 animals were injected with saline, 10 ml/kg, and 3 with macusine B, 10 mg/kg i.p. Each animal acted as its own control and therefore on the second day of the experiment the rats were injected with either saline or macusine B.

EFFECT ON THE CONDITIONED AVOIDANCE RESPONSE OF RATS

Some 4 days before the start of the experiment, 5 rats were trained to jump over a barrier at the sound of an electric doorbell. This was an

avoidance reaction to the electric shock which was applied to the feet 5 sec after the bell should the animal fail to show the positive response. Two min later the bell was rung again and this was repeated for each rat 10 times per daily run. Three of the rats conditioned easily and gave approximately 9 positive responses by the fourth day of the training schedule, but 2 of the rats only gave 3 or 4 positive responses when subjected to the same schedule. Each day of the training period the animals were injected with saline (10 ml/kg i.p.) 10–15 min before being put into the conditioning cage. On the 5th day, all the animals were injected with macusine B (10 mg/kg i.p.) and the experiment was repeated.

EFFECT ON THE KNEE JERK REFLEX OF THE CAT

Two cats were anaesthetised with chloralose (100 mg/kg i.p.), the common carotid artery was cannulated and the knee jerk was elicited by an electrically driven hammer once every 10 sec. Macusine B (0·5–1·0 mg/kg) was injected into the femoral vein and the effects on the blood pressure and knee jerk reflex were recorded.

Results and discussion

Macusine B significantly reduced the induction time and increased the sleeping time tc barbiturate anaesthesia in mice. The induction time was 5·81 $\pm$ 0·64 (standard error) min for the control group and 4·08 $\pm$ 0·04 min for the experimental group which had been injected with macusine B at the same time as the barbiturate ($P < 0{\cdot}001$). There was a similar reduction in the induction time for anaesthesia between the control group and the other experimental groups in which macusine B was found to potentiate the sleeping time. The potentiation of the sleeping time was found to be greatest (280% of the control value) in the group of mice which had been treated 4 hr previously with macusine B, but there was no significant difference between the group of mice pretreated with the alkaloid for 13 hr and the control group (Fig. 1).

In the previous investigation of macusine B (Leonard, 1965) it was found that the alkaloid had marked convulsant properties and it was therefore of interest to determine in what way this alkaloid potentiated the barbiturate sleeping time. Mullen & Fouts (1965) in their investigation of several adrenergic blocking drugs found that those compounds potentiating the barbiturate sleeping time of mice did so either indirectly, by causing vasodilatation and hence hypothermia, or directly by blocking the breakdown of the barbiturate by the liver microsomes. To determine whether mascusine B, which also blocks some adrenergic receptors, potentiated the sleeping time in one of these ways, the effect of this alkaloid was investigated on the rectal temperature of mice under barbiturate anaesthesia. When the mice were at an ambient temperature of 20° the experimental group had a significantly lower rectal temperature than the control group, this difference being first apparent 15 min after injection of macusine B (Fig. 2). There was also a significant increase in the sleeping time of the experimental group compared with the control

group. However, when the experimental and control groups were kept at an ambient temperature of 35° the difference between the rectal temperatures and the sleeping time of the control (26·4 ± 1·5 min) and the experimental group (22·4 ± 0·5 min) was not significant. These results are therefore consistent with the view that macusine B potentiates the barbiturate sleeping time as a consequence of hypothermia and in this respect is comparable with another α-adrenergic blocking drug tolazoline which Mullen & Fouts (1965) found to potentiate the sleeping time in a similar way.

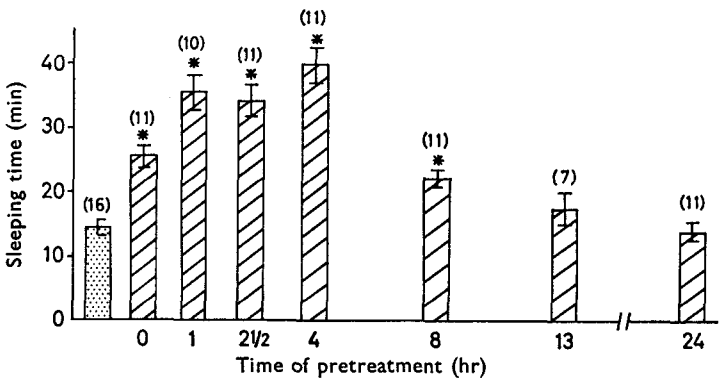

FIG. 1. Effect of macusine B on the barbiturate sleeping time of mice. Groups of mice injected with amylobarbitone sodium alone as controls (area stippled) or with amylobarbitone and macusine B (25 mg/kg) (areas hatched). The latter were pretreated with macusine B at the times indicated before injection of the barbiturate. The histograms represent the mean sleeping time ± standard error for each group. The number of animals per group is given in parentheses. * Difference between the experimental and control group significant at $P < 0.001$ level.

The structure of macusine B and the similarity of its pharmacological properties to the ergot alkaloids (Leonard, 1965) suggests that it may have some effect on the synthesis, breakdown or binding of amines within the brain. The results of the experiments in which 5-HT, γ-aminobutyric acid, noradrenaline and dopamine were measured in the brain, and noradrenaline and dopamine in the rat heart, following the injection of macusine B, showed that this alkaloid had no apparent effect on the levels of these amines irrespective of the time of pretreatment with the alkaloid. Furthermore, when rats were injected with reserpine and macusine B, 4 hr before killing, there was no significant difference between the extent of the depletion of 5-HT and noradrenaline in the brain of the animals injected with reserpine alone (5-HT was 66% and noradrenaline was 40% of the control value) and the animals injected with macusine B and reserpine (5-HT was 60% and noradrenaline was 34% of the control value). These results suggest that, at least at the dose level used, macusine B does not affect the synthesis, binding or breakdown of these amines in the rat brain. The possibility exists that the alkaloid has a direct action on central adrenergic receptors and one method of investigating this possibility is by the effect of the alkaloid on the hyperactivity induced in mice by (±)-amphetamine. Preliminary results from this laboratory show that

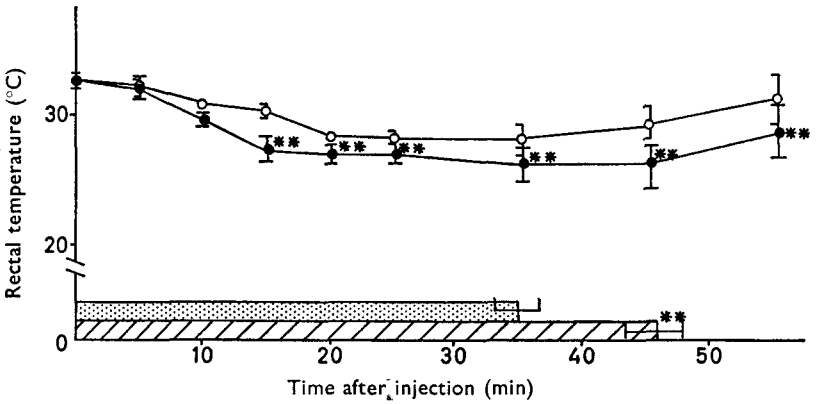

FIG. 2. Effect of macusine B on the rectal temperature of mice under barbiturate anaesthesia. Ambient temperature 20°. Groups of 12 mice injected with amylobarbitone sodium alone (○) or with amylobarbitone sodium and macusine B (10 mg/kg) (●). Each point represents the mean ± standard error. ** Difference between the experimental and control group significant at $P < 0{\cdot}05 > 0{\cdot}02$. The sleeping time of the control (area stippled) and experimental groups (area hatched) indicated in the histograms.

mascusine B partially blocks the amphetamine-induced hyperactivity suggesting that it has some action on central adrenergic receptors. In this respect, macusine B resembles the action of such α-adrenergic blocking drugs as tolazoline which have a similar effect on the amphetamine-induced hyperactivity (Tripod, 1952). Nickerson (1959) has suggested that one of the difficulties of attributing these effects of tolazoline to a specific central adrenergic blockade is that the effect is not quantitatively correlated with the peripheral adrenergic blocking activity, so that caution must be shown in attributing the blocking of the amphetamine induced hyperactivity by macusine B to a specific action on central adrenergic receptors.

On many occasions during the investigation of the general pharmacology of macusine B it was observed that rats and mice became behaviourally depressed when injected with subconvulsive doses of the alkaloid. As a result of this observation, the general activity of rats was measured over the 24 hr period after the injection of the alkaloid. The results show that animals injected with the alkaloid had a significantly lower activity over the first 12 hr period (226 ± 3·2 counts/12 hr) compared with the control animals (287 ± 7·8 counts/12 hr; $P < 0{\cdot}05 > 0{\cdot}02$). However there was no significant difference in the activity of these groups during the second 12 hr period of the experiment. From this it seems reasonable to conclude that the slight depression in activity produced by subconvulsive doses of the alkaloid was possibly due to hypothermia, but, in order to exclude the possibility of macusine B having any tranquillising activity, its effect on the conditioned avoidance response of rats was tested. The rats showed a total of 33 positive responses to the auditory stimulus after the fourth day of training. On the fifth day, after the same rats had been

injected with a subconvulsive dose of macusine B, the animals also gave a total of 33 positive responses to the stimulus. This experiment indicates that macusine B does not produce the depression in behaviour activity by a tranquillising action and therefore presumably produces this effect as a consequence of hypothermia.

In the previous investigation of the pharmacology of macusine B it was found that this alkaloid did not have any apparent effect on the neuromuscular junction *in vitro* even when present in high concentrations. However in the present study, when macusine B was injected *in vivo*, it was found to produce a transient depression of the knee jerk reflex accompanied by an appreciable fall in the blood pressure (approximately 40 mm mercury). When lower doses of the alkaloid were injected which did not affect the blood pressure, the knee jerk reflex was also unaffected. This seems to suggest that macusine B depressed this reflex as a consequence of its effect on the blood pressure. The general conclusion can also be reached that although macusine B is a convulsant alkaloid not structurally dissimilar to strychnine, it is evident from these studies that it is quite unlike strychnine in its pharmacological activities.

In conclusion, macusine B resembles other adrenergic blocking drugs in its pharmacological activities and, apart from its convulsant activity, it seems likely from the present investigation that its actions on the central nervous system are a consequence of its effect on the vascular system. It is possible that the alkaloid may have an effect on specific regions of the brain which are unlikely to be discovered by the methods used in the present study. It is of interest in this respect to find that lysergic acid diethylamide, while having little apparent effect on many of the conventional pharmacological screening tests used to investigate the actions of drugs on the central nervous system, is an inhibitor of cortical synapses (Marrazzi & Hart, 1955), a potent hallucinogen and furthermore does inhibit some peripheral (Hornykiewicz & Obenaus, 1958) and central (Goldstein, 1962) adrenergic receptors.

Acknowledgements. The author wishes to thank Professor A. R. Battersby for providing the sample of macusine B.

References

Anton, A. H. & Sayre, D. F. (1962). *J. Pharmacol.*, **138**, 360–375.
Anton, A. H. & Sayre, D. F. (1964). *Ibid.*, **145**, 326–336.
Bogdanski, D. F., Pletscher, A., Brodie, B. B. & Udenfriend, S. (1956). *Ibid.*, **117**, 82–88.
Goldstein, L. (1962). *Fed. Proc.*, **21**, 337.
Hornykiewicz, O. & Obenaus, H. (1958). *Wien. klin. Wschr.*, **70**, 948–9.
Leonard, B. E. (1965). *J. Pharm. Pharmacol.*, **17**, 566–576.
Leyden, A. F., Pomerantz, E. & Bouchard, E. F. (1956). *J. Amer. pharm. Ass., Sci. Ed.*, **45**, 771–775.
Lowe, I., Robins, E. & Eyerman, G. S. (1958). *J. Neurochem.*, **3**, 8–18.
Marrazzi, A. S. & Hart, E. R. (1955). *Electroenceph. clin. Neurophysiol.*, **7**, 146.
Mullen, J. O. & Fouts, J. R. (1965). *Biochem. Pharmacol.*, **14**, 305–311.
Nickerson, M. (1959). *Pharmacol., Rev.*, **11**, 443–461.
Shore, P. A. & Olin, J. S. (1958). *J. Pharmacol.*, **122**, 295–300.
Tripod, J. (1952). *Helv. physiol. acta.*, **10**, 403–412.
Uchida, T. & O'Brien, R. D. (1964). *Biochem. Pharmacol.*, **13**, 725–730.