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An investigation of the pharmacology of macusine B

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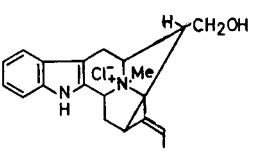
Macusine B, an alkaloid isolated from *Strychnos toxifera*, blocks α-adrenergic receptors and stimulates β-receptors *in vivo* and *in vitro*. It partially blocks the response of the isolated rabbit heart and the cat blood pressure to tyramine. Macusine B is a competitive inhibitor of 5-hydroxytryptamine on the guinea-pig ileum and also blocks the action of this spasmogen on the rat uterus.

THE first recorded observation of the pharmacological potency of the curare obtained from *Strychnos toxifera* was made by Schomburgk (1879) who reported that the arrow poison prepared from this plant by the Macusi tribe of British Guiana was the most potent hitherto observed. Since then over 60 alkaloids have been found in *S. toxifera* (Battersby, Binks, Hodson & Yeowell, 1960). Toxiferine I was one of the first crystalline alkaloids to be isolated from this plant and the potency of the arrow poison was largely due to the presence of this alkaloid. Herring & Marsh (1951) and Paton & Perry (1951) were the first to investigate the powerful neuromuscular blocking action of toxiferine I.

Because of the difficulty involved in the isolation and purification of the alkaloids from *S. toxifera* few of the other alkaloids from this species have undergone pharmacological investigation; Battersby & others (1960) isolated and identified the alkaloids macusine A and B and have since obtained sufficient macusine B for pharmacological investigation. The alkaloid has the structure



It is a white crystalline solid which is readily soluble in water to give a neutral solution.

Experimental

METHODS

Effect on the whole animal. Ten female albino mice (16–20 g) were injected with an approximately LD50 dose (50 mg/kg i.p.) of macusine B and observed for at least 15 min. During this time the mice were periodically tested on a rotating rod and also for their ability to grip a rough surface. The time of onset of any effect of macusine B was noted. For the LD50 determination, female albino mice (16–20 g) were used. After estimating the approximate LD50, four groups of 10 mice were injected intraperitoneally with doses of the alkaloid in a logarithmic series of 0·25, 0·50, 1·0 and 2·0 respectively where 1·0 was the approximate LD50. The LD50 was estimated by the method of Weil (1953).

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Guinea-pig isolated ileum. The ileum was suspended in a 15 ml bath of oxygenated Tyrode solution at 37°. Longitudinal contractions were recorded isotonically. The lever was weighted with 0·5 g and had a $\times 10$ magnification. Submaximal contractions were induced to constant doses of acetylcholine, histamine, barium chloride, nicotine and 5-hydroxytryptamine (5-HT), and when a steady response had been achieved for each agonist macusine B was added to the bath and the effects on the responses to the subsequent doses of the agonist were recorded. The method of Timms (1956) was used to test for the type of inhibition produced by the alkaloid.

Rat isolated colon. The terminal colon was suspended in a 15 ml bath of oxygenated De Jalon solution at 30° as described by Gaddum & Lembeck (1949). The effect of macusine B on the depressor effect of adrenaline on the acetylcholine-stimulated contractions was then observed.

Rat isolated uterus. Virgin rats (90–110 g) were injected subcutaneously with stilboestrol propionate (1 mg/kg) 24 hr before killing. The uteri were suspended in a 15 ml bath of oxygenated De Jalon fluid at 30° as described by Gaddum & Lembeck (1949). The effect of macusine B on the submaximal contractions caused by 5-HT and vasopressin was observed. In other experiments the uterus was stimulated with a standard dose of carbachol and the effect of macusine B on the depressor response of adrenaline on the carbachol-stimulated uterus was observed.

Rat phrenic nerve-diaphragm. This was suspended in a 15 ml bath of oxygenated Tyrode solution at 37° as described by Bülbring (1946). Macusine B was added to the bath and its effect observed on the contractions of the diaphragm that followed the electrical stimulation of the phrenic nerve.

Rat heart rate and electrocardiogram (ECG). Rats (90–110 g) were anaesthetised with pentobarbitone sodium (60 mg/kg; i.p.), after 15 min, the resting ECG was recorded following an intraperitoneal injection of physiological saline (0·1 ml). Macusine B was injected (15 mg/kg, i.p.) and a record made for 30 sec periods immediately after injection and subsequently after periods of 1, 2, 3, 4, 5, 7 and 10 min.

Guinea-pig isolated vas deferens—hypogastric nerve. The tissue was set up in a 50 ml bath of oxygenated Tyrode solution as described by Birmingham & Wilson (1963). The effect of macusine B on the contractions of the vas deferens was observed following stimulation of the pre-ganglionic nerve.

Chick isolated rectum. Approximately 6 cm of rectum from 14–21 day old chicks was suspended in a 15 ml bath of Krebs solution as described by Armitage & Vane (1964). The direct effect of macusine B on the chick rectum was recorded in addition to its effects on the responses of the rectum to adrenaline. The response of the isolated rectum to adrenaline alone was also observed.

Rabbit isolated duodenum. A piece of duodenum was suspended in a 50 ml bath of oxygenated Tyrode solution at 37° and the effect of macusine B on the muscle tone and pendular movement was observed.

Rabbit isolated heart. The rabbit heart was perfused with oxygenated

Ringer solution at 37° by the method of Langendorff. The effect of macusine B on the heart rate and amplitude of contraction was tested at first alone and then following the injection of tyramine and adrenaline into the perfusion fluid.

Test for local anaesthesia. The frog lumbar plexus anaesthesia method of Bülbring & Wajda (1945) was used. The determinations were made using three groups of 5 frogs. The first group received a 0·2% solution of macusine B in 0·65% saline intraperitoneally, the second group was given a 0·2% solution of lignocaine hydrochloride in 0·65% saline and the control group received 0·65% saline alone. The local anaesthetic potencies of macusine B and lignocaine were compared by observing the times taken for the frogs to fail to withdraw their legs from a beaker of dilute hydrochloric acid.

Cat blood pressure. Cats (2–4 kg) were anaesthetised with chloralose (80 mg/kg, i.p.) and the blood pressure was recorded from the carotid artery. The nictitating membrane was attached to a frontal writing level giving a ×15 magnification and the preganglionic fibres of the sympathetic chain were stimulated by means of supramaximal shocks of 10 sec duration at 1 min intervals from a Palmer stimulator.

The effect of macusine B on the normal blood pressure, and its effect on the pressor response to adrenaline, noradrenaline, tyramine, carotid occlusion for 10 sec, and on the depressor response to acetylcholine, vagal stimulation of 5 sec duration and carotid occlusion for 10 sec was observed. All drugs were injected into the femoral vein.

Blood glucose determination. Blood was obtained from mice by making a small incision in the lateral tail vein after first dipping the tail into warm water. Serial samples of blood (0·05–0·1 ml) could readily be obtained in this way. Three groups of 5 mice were used. Group 1 was injected subcutaneously with adrenaline (1 mg/kg); group 2 was injected with the same dose of adrenaline together with macusine B (25 mg/kg, i.p.); group 3 was given the alkaloid (25 mg/kg, i.p.) alone. The control group was injected with physiological saline (0·1 ml, s.c.). The blood glucose was estimated by the glucose oxidase method (Huggett & Nixon, 1956).

Plasma non-esterified fatty acids. Four groups of 4 rats were used. Group 1 was given adrenaline (1 mg/kg, s.c.); group 2 was injected with the same dose of adrenaline together with macusine B (15 mg/kg, i.p.); group 3 was given macusine B (15 mg/kg, i.p.) alone. The control group was injected with physiological saline (0·1 ml, s.c.). All animals were killed by a blow on the head 3 hr after injection; this corresponded approximately to the period of peak activity for adrenaline as assessed in a preliminary experiment. Plasma free fatty acids were then determined by the method of Duncombe (1964).

Results

Effect on whole animal. By 3½ min after the injection of an approximate LD50 dose of macusine B, the mice showed a slight head tremor, followed shortly by ataxia and a reduced grip. The ability of the animals to hold onto a rotating rod was completely lost 5–6 min after injection. Clonic

convulsions occurred after approximately 7½ min and loss of righting reflex, cyanosis and death resulted within 9 min. The mice in the group surviving this dose showed only the first two phases of the seizure. The LD50 on intraperitoneal injection was: 53·7 mg/kg with 95% confidence limits of 42·8–67·2.

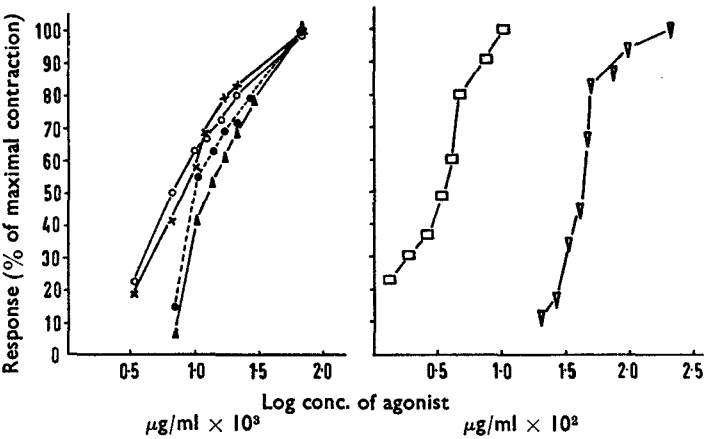

FIG. 1. The effect of macusine B (1·3 μg/ml) on the responses of the guinea-pig isolated ileum to acetylcholine (×, ○), histamine (▲, ●) and 5-hydroxytryptamine (□, ▽). The symbols ×, ▲ and □ represent the responses to the agonists; the others represent the responses after equilibration with macusine B. Each curve represents the mean of two experiments.

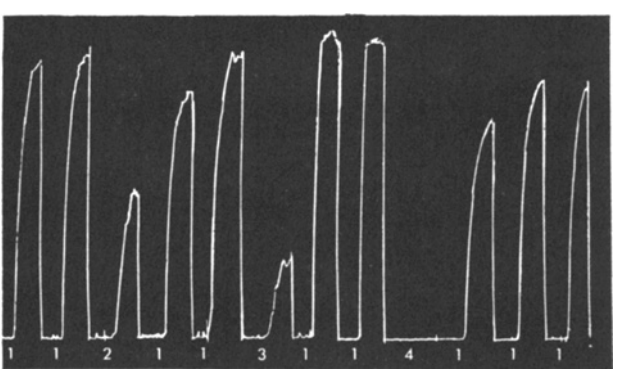

FIG. 2. Effect of macusine B on the responses of the guinea-pig isolated ileum to nicotine. (1) 1·33 μg/ml nicotine hydrogen tartrate, (2) 1·33 μg/ml nicotine + 1·66 μg/ml macusine B, (3) 1·33 μg/ml nicotine + 3·32 μg/ml macusine B, (4) 1·33 μg/ml nicotine + 6·64 μg/ml macusine B.

Guinea-pig isolated ileum. Macusine B had no effect on the contractions caused by the action of acetylcholine, histamine and barium chloride on this preparation. It did inhibit the responses of the ileum to 5-HT, and the parallel displacement of the dose-response curve to the right (Fig. 1) suggests that the alkaloid is competitively inhibiting 5-HT. Macusine B also reversibly inhibited the responses of the ileum to nicotine (Fig. 2).

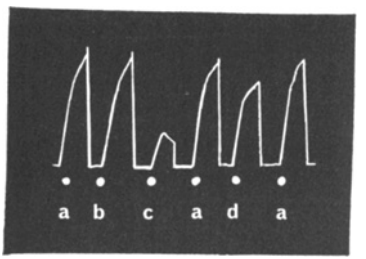

FIG. 3. Effect of macusine B on the rat colon. Colon suspended in oxygenated De Jalon solution at 30°. Response of colon shown to (a), 0·01 μg/ml acetylcholine (as chloride); (b), 0·01 μg/ml acetylcholine + 1·70 μg/ml mascusine B (as hydrochlorine); (c), 0·01 μg/ml acetylcholine + 0·01 μg/ml adrenaline (as hydrogen tartrate); (d), 0·01 μg/ml acetylcholine + 1·70 μg/ml macusine B + 0·01 μg/ml adrenaline.

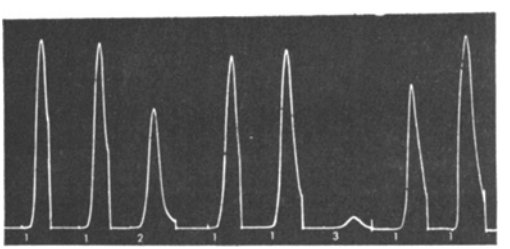

FIG. 4. Effect of macusine B on the responses of the rat isolated uterus to 5-HT. (1) 0·033 μg/ml 5-HT creatine sulphate, (2) 0·033 μg/ml 5-HT + 1·66 μg/ml macusine B, (3) 0·033 μg/ml 5-HT + 6·64 μg/ml macusine B.

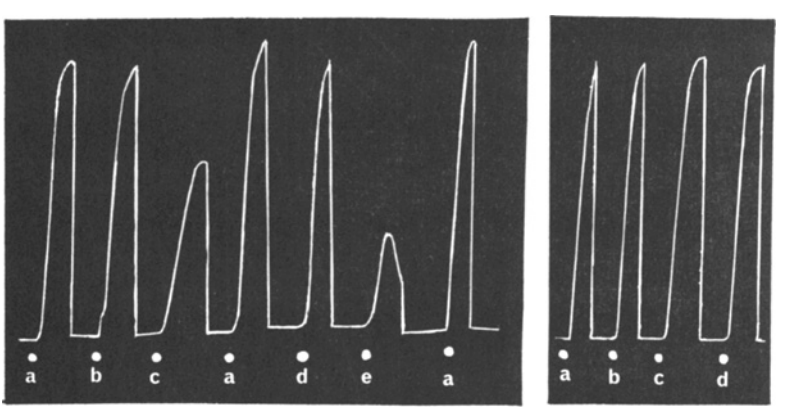

FIG. 5. Effect of macusine B on the rat uterus stimulated with carbachol. Response of uterus shown to (a), 0·66 μg/ml carbachol; (b), 0·66 μg/ml carbachol + 1·30 μg/ml macusine B; (c), 0·66 μg/ml carbachol + 0·01 μg/ml adrenaline; (d), 0·66 μg/ml carbachol + 1·3 μg/ml macusine B; (e), 0·66 μg/ml carbachol + 1·3 μg/m macusine B + 0·01 μg/ml adrenaline. Second trace shows response to (a), 0·66 μg/ml carbachol; (b), 0·66 μg/ml carbachol + 1·0 μg/ml pronethalol; (c), 0·66 μg/ml carbachol + 0·01 μg/ml adrenaline; (d), 0·66 μg/ml carbachol + 1·3 μg/ml macusine B + 0·01 μg/ml adrenaline.

Rat isolated colon, uterus and phrenic nerve diaphragm. Macusine B partially blocked the depressant effect of adrenaline on the acetylcholine-stimulated colon (Fig. 3) and this effect was easily reversible. The alkaloid blocked the response of the uterus to 5-HT (Fig. 4). The effect was readily reversible. It had no effect on the responses of the uterus to acetylcholine or vasopressin.

In a dose of 1·3 μg/ml, the alkaloid potentiated the inhibitory effect of adrenaline on the carbachol-stimulated uterus (Fig. 5). This effect was readily reversed by washing. The inhibitory effect of macusine B was blocked by the β-receptor blocking drug pronethalol (Black & Stephenson, 1962).

The alkaloid had no effect on the electrically stimulated contractions of the diaphragm when doses of up to 80 μg/ml were added to the bath.

Rat heart rate and ECG. Macusine B had no effect on the ECG of the rat in sublethal doses other than to cause a small increase in the heart rate. This increase began 1 min after the intraperitoneal injection of the alkaloid and lasted for approximately 3 min.

Guinea-pig vas deferens—hypogastric nerve. Macusine B blocked the electrically stimulated contractions of the vas deferens in a dose of 10 μg/ml. The effect was reversible.

Rabbit isolated duodenum and heart. Macusine B reduced the muscle tone when added to the bath in a dose of 0·5 μg/ml (Fig. 6), and this

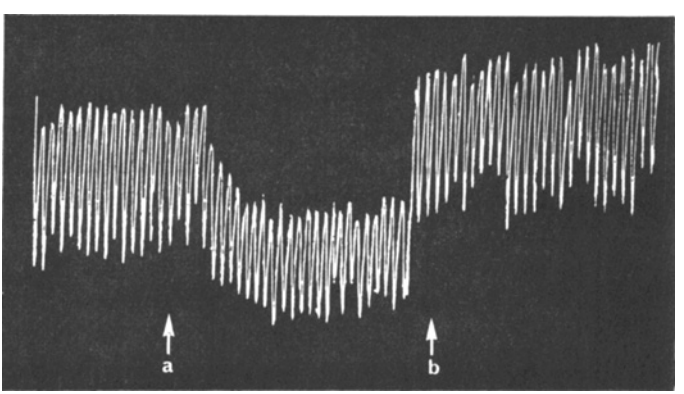

FIG. 6. Effect of macusine B on the rabbit duodenum. Duodenum suspended in oxygenated Tyrode solution at 37°. Response of duodenum to 0·5 μg/ml macusine B shown. Alkaloid added at (a) and the bath washed out at (b).

effect was easily reversible. The alkaloid had no effect on the pendular movement of the duodenum and at 2·5 μg affected neither the amplitude of contraction nor the heart rate when injected into the perfusion fluid. However, it did block the effect of tyramine on the isolated heart (Fig. 7) and slightly potentiated the increase in the heart rate due to an injection of 0·5 μg of adrenaline. The alkaloid did not affect the increase in amplitude of contraction of the isolated heart caused by adrenaline.

Chick isolated rectum. Macusine B caused a decrease in the muscle tone and also potentiated the depressor effect of adrenaline on this preparation, when added to the organ bath in a dose of 1·3 μg/ml. When added to the bath alone, this concentration of alkaloid was equiactive with 0·005 μg/ml of adrenaline.

Test for local anaesthesia. There was no significant difference between the group treated with macusine B and the control group. The time for the onset of plexus anaesthesia for the experimental and the control group

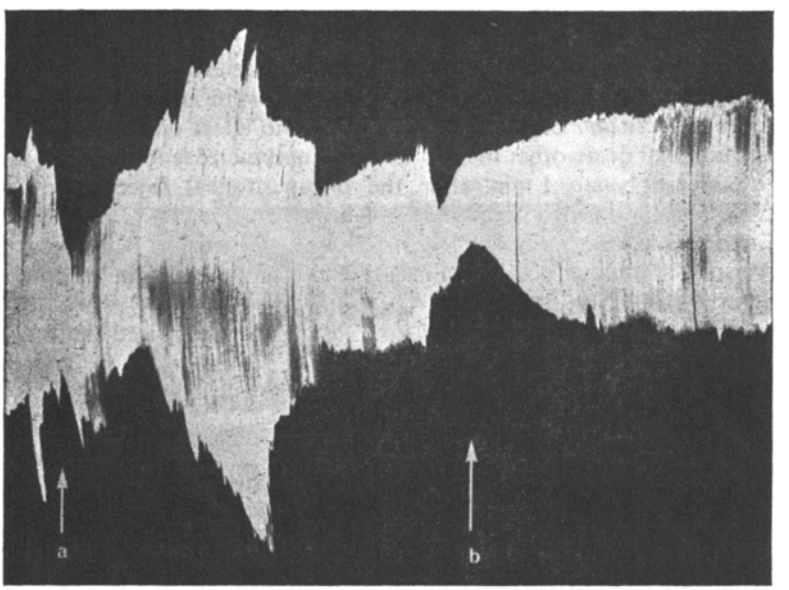

FIG. 7. Effect of macusine B on the rabbit heart. Heart perfused with oxygenated Ringer solution at 37°. At (a) 20 μg tyramine (as hydrochloride) added to perfusion fluid and at (b) 20 μg tyramine + 25 μg macusine B added.

was 14·0 $\pm$ 0·35 min ($\pm$ s.e.) and 13·5 $\pm$ 0·50 min respectively. In contrast, the time of onset of anaesthesia for the group treated with 0·2% lignocaine was 5·2 $\pm$ 0·77 min.

Cat blood pressure. Macusine B had a marked hypotensive effect which was readily blocked by pronethalol. The alkaloid also reversed the pressor response to adrenaline and in most instances completely blocked the pressor response to noradrenaline, but only partially blocked that due to tyramine. These effects lasted for up to 30 min after intravenous injection. Pronethalol blocked the effect of macusine B on the pressor response to adrenaline, noradrenaline and tyramine, but approximately 45 min after the administration of pronethalol it was found that, although the hypotensive response to macusine B was still blocked, the alkaloid could once more reduce the pressor response to adrenaline. In the experiments in which the electrocardiogram was recorded, macusine B alone was found to increase the heart rate for 5–10 min after injection.

In some experiments the alkaloid not only reversed the effect of adrenaline but also reversed that of noradrenaline. This was repeated several times on 3 of the 8 cats showing the noradrenaline reversal.

Macusine B did not affect the electrically stimulated contractions of the nictitating membrane. It also had no effect on the depressor response to acetylcholine and vagal stimulation nor on the pressor response which follows the occlusion of the carotid arteries for 10 sec.

In experiments in which the respiration was also recorded, the alkaloid depressed the respiratory rate and caused irregular breathing.

Blood glucose and plasma non-esterified fatty acids. Macusine B in a dose of 25 mg/kg (approximately half of the LD50 dose), completely blocked the hyperglycaemic effect of adrenaline. The alkaloid had only a slight effect on the blood glucose level when injected alone (Fig. 8).

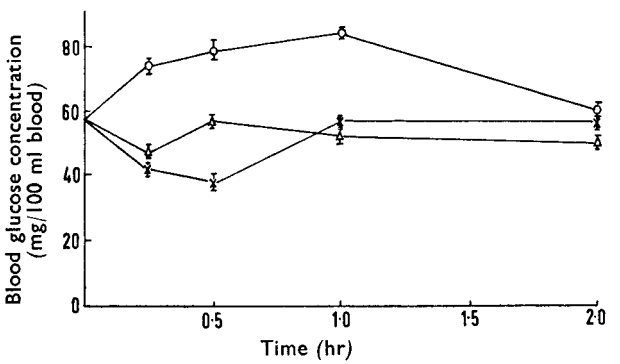

FIG. 8. Effect of macusine B on the blood glucose level of mice. Mice injected with 1 mg/kg adrenaline (s.c.). O; 25 mg/kg macusine B (i.p.) alone △ and 1 mg/kg adrenaline + 25 mg/kg macusine B (i.p.) ×. Blood samples withdrawn at 15, 30, 60 and 120 min after injection. Each point shows the mean ± standard error.

It significantly reduced the rise in plasma free fatty acids caused by the subcutaneous injection of adrenaline (Table 1). The dose of alkaloid used had no effect on the plasma free fatty acid level of the group injected with the alkaloid alone.

TABLE 1. EFFECT OF MACUSINE B ON THE NON-ESTERIFIED FATTY ACID LEVEL OF RATS

Group	NEFA μ-equiv./litre
Controls	535 ± 7·2* (7)
Adrenaline alone	725 ± 37† (4)
Macusine B alone	531 ± 40 (4)
Adrenaline + macusine B ..	567 ± 9·3 (4)

Controls given saline (i.p.). Groups given adrenaline alone (1 mg/kg s.c.), macusine B alone (15 mg/kg i.p.) or adrenaline (1 mg/kg s.c.) and macusine B (15 mg/kg i.p.).

* Mean ± standard error.

† Difference from controls significant ($P \geqslant 0{\cdot}001$). Number of animals in each group indicated n parentheses.

Discussion

Structurally, macusine B resembles the ergot and rauwolfia alkaloids and the toxiferines, but its pharmacological activity more closely resembles that of the ergot alkaloids than that of the other groups. It is probable

that because of the high toxicity of toxiferine I, the activity of macusine B has been masked in previous investigations of the crude extracts of *S. toxifera.*

From this study it appears that the main effect of macusine B is on both α- and β-adrenergic receptors. The α-adrenergic-blocking effect of the alkaloid is suggested by its reversal of the pressor response to adrenaline and to its blocking the pressor response to noradrenaline in the cat. This effect is further suggested by its blocking the action of adrenaline on the acetylcholine-stimulated contractions of the rat colon. The β-sympathomimetic effect of macusine B is suggested by the fact that its hypotensive action is blocked by pronethalol. Since the alkaloid has no direct effect on the isolated heart, the hypotension is presumably due to vasodilatation in the vascular bed. Further evidence for the sympathomimetic effect is provided by the observation that the alkaloid relaxes the rabbit isolated duodenum and chick rectum and potentiates the depressor effect of adrenaline on the carbachol-stimulated rat uterus, an action blocked by pronethalol.

Macusine B blocks the action of tyramine on the rabbit isolated heart and this can explain the reduction in the pressor response to tyramine by the alkaloid *in vivo.* Burn & Rand (1958) suggested that tyramine causes its pressor response by liberating noradrenaline from endogenous stores and therefore the alkaloid could partially block the tyramine response *in vivo* by blocking the α-adrenergic receptors. In doing this, macusine B resembles the action of the ergot alkaloids (Swaine, 1963). There is now some evidence to suggest that tyramine has a direct sympathomimetic action which is independent of its ability to release noradrenaline (Vane, 1960; Nasmyth, 1962; Varma, Gillis & Benfrey, 1964) and therefore the effect of macusine B on the tyramine response may not be due entirely to its α-adrenergic blocking action.

Macusine B in a dose that causes hypotension does not depress the electrically stimulated contractions of the nictitating membrane. Furthermore it only depresses the contractions of the electrically stimulated guinea-pig vas deferens-hypogastric nerve preparation in a dose which is approximately eight times greater than any that is effective on other isolated organ preparations. It seems unlikely therefore that the alkaloid causes hypotension by ganglionic blockade.

The metabolic effects of adrenaline have been variously ascribed to stimulation of both the α- and β-adrenergic receptors or to the activation of some other type of receptor (Furchgott, 1959). If the glycogenolytic action of adrenaline can be explained in terms of the stimulation of α-receptors then it is apparent that macusine B blocks the hyperglycaemic effect of adrenaline by blocking these receptors and in so doing resembles the action of other compounds which block the α-adrenergic receptors. Thus Levi & McCutcheon (1964) in their study of the effect of several sympathomimetic amines and adrenergic blocking drugs, found that the hyperglycaemia and hyperlacticacidaemia caused by adrenaline was mainly due to stimulation of the α-receptors and that stimulation of the β-receptors had a much smaller effect. Similar results were reported

by Sutherland & Cori (1948) and Ellis (1956), while Ellis, Anderson & Collins (1953) found that dihydroergotamine effectively blocked the glycogenolytic acitvity of adrenaline *in vitro*. Nevertheless, these findings are in disagreement with those of Van der Pol (1956), McCutcheon (1962), Pilkington, Lowe, Robinson & Titterington (1962) and Hornbrook & Brody (1963) who showed that the glycogenolytic effects of adrenaline can be inhibited mainly by blocking the β-receptors.

Besides blocking the hyperglycaemic effect of adrenaline, macusine B also inhibits its lipid mobilising effect. Since the alkaloid only blocks the α-receptors it seems likely that the hyperlipaemia produced by adrenaline is due to the stimulation of the α-receptors, as has been suggested by Gordon & Cherkes (1956) and Jeanrenaud (1961) and not by the stimulation of the β-receptors as has been reported by Love, Caar & Ashmore (1963). Further evidence for the view that lipid mobilization is not due to stimulation of the β-receptors is provided by the observation that macusine B does not affect the plasma lipid levels when injected alone, even though it stimulates the β-receptors in several isolated organ and whole animal preparations. The seemingly irreconcilable findings reported in the literature for the type of receptor upon which adrenaline acts to produce its metabolic effects might be due to differences in the species or strain of animals used, to the metabolic effects being mediated by different receptors under different physiological conditions or in some way not involving receptors.

Macusine B is a competitive inhibitor of 5-HT on the guinea-pig isolated ileum, yet it reversibly inhibits the responses of the ileum to nicotine, which suggests that it is not a specific antagonist of 5-HT. Gaddum & Hameed (1954) suggested that 5-HT acts on specific receptors in the guinea-pig isolated ileum which are distinct from the nicotine receptors. Later, Gaddum & Picarelli (1957) advanced evidence suggesting that there were two types of tryptamine receptor in the terminal ileum of the guinea-pig, one type being associated with smooth muscle (D-receptor) and the other type associated with the ganglia (M-receptor). This view has been challenged by the investigations of Day & Vane (1963), who found that the effect of 5-HT on the guinea-pig ileum was primarily due to the agonist stimulating the receptors in the nervous tissue while the receptors associated with the smooth muscle were of little significance in eliciting the usual response. Brownlee & Johnson (1963) also showed that 5-HT contracted the ileum by activating receptors situated in the intramural parasympathetic ganglion cells and that these receptors were pharmacologically distinct from those activated by dimethylphenylpiperazinium and nicotine. It is possible that macusine B may inhibit the responses of the ileum to these agonists by acting on ganglia and that it may also have ganglion blocking or local anaesthetic activity but so far no direct evidence has been found for either activity in concentrations used to produce the pharmacological effects already described. The action of macusine B on the ganglia therefore resembles that of another α-adrenergic blocking drug phenoxybenzamine which inhibits the responses of the ileum to 5-HT and nicotine (Brownlee & Johnson, 1963). However,

the mode of action of macusine B and phenoxybenzamine on the responses of the ileum to these agonists must differ, for, whereas phenoxybenzamine depresses the responses to nicotine and 5-HT at concentrations which also depress the responses to acetylcholine and histamine (Brownlee & Johnson, 1963), macusine B only inhibits the 5-HT and nicotine induced responses. Macusine B also inhibits the effect of 5-HT on the isolated rat uterus presumably by acting on the tryptamine receptors which occur on the smooth muscle. Thus macusine B, while resembling the action of some of the ergot alkaloids in blocking the 5-HT—induced responses of the rat uterus, differs from these alkaloids in its ability to block the 5-HT and nicotine elicited responses of the guinea-pig ileum.

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