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VASOPRESSIN RELEASE PRODUCED IN ANAESTHETIZED CATS BY ANTAGONISTS OF γ-AMINOBUTYRIC ACID AND GLYCINE

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1 In cats anaesthetized with chloralose, the central excitatory substances, tubocurarine, picrotoxin, bicuculline, leptazol and strychnine, were applied to the exposed ventral surface of the brain stem through paired Perspex rings placed across the medulla and their effects on vasopressin release and arterial blood pressure were examined.

2 The excitatory substances released large amounts of vasopressin when applied to an area 6–9 mm caudal to the trapezoid bodies. From this area vasopressin release was previously obtained with nicotine.

3 With nicotine, the vasopressin release occurred almost instantaneously and tachyphylaxis developed rapidly. With the excitatory substances the release increased gradually and there was no tachyphylaxis. When these substances were applied for several minutes, the release reached its maximum a considerable time after their removal, except with leptazol when release diminished at once after removal.

4 The excitatory substances had little or no effect on arterial blood pressure when applied to the vasopressin releasing area, but produced strong pressor responses when applied to a more rostrally situated area.

5 It is concluded that the excitatory substances release vasopressin and raise arterial blood pressure because they are antagonists of γ-aminobutyric acid and/or glycine and that numerous inhibitory neurones which release these amino-acids synapse at the ventral surface of the medulla. The physiological function of those which synapse at the vasopressin releasing area may be to act as a brake on vasopressin release, and of those which synapse at the more rostrally situated area to act as a brake on arterial blood pressure.

Introduction

Recently it was shown that nicotine injected into the cerebral ventricles of anaesthetized cats caused release of large amounts of vasopressin into the blood stream, but the effect did not result from an action on structures in the ventricular walls. The nicotine had first to pass into the subarachnoid space. From there it acted on structures situated bilaterally at the ventral surface of the medulla oblongata (Bisset, Feldberg, Guertzenstein & Rocha e Silva Jr, 1975).

This surface is known to contain two areas from which blood pressure effects can be obtained, a more rostrally and a more caudally situated one (Feldberg & Guertzenstein, 1976). Both areas have been delineated by applying drugs through paired Perspex rings placed across the medulla at different positions. The upper limits of the areas covered by the rings were found to lie, for the rostral region, just caudal to the trapezoid bodies, and for the caudal area, 5–6 mm caudal to them. In the insets of Figures 6 and 7, the two areas are indicated, the rostral area by the ovals A, the caudal area by the ovals B. The vasopressin release produced by nicotine was obtained only from area B.

It seemed unlikely that nicotine should be the only substance having this property. A search was therefore made for other drugs which might have a similar action, and it was found that the central excitatory substances tubocurarine, picrotoxin, bicuculline, leptazol and glycine, shared this property with nicotine.

For nicotine it was shown that the release of vasopressin occurred without the release of oxytocin. Whether this applies also to the central excitatory substances has not been examined, nor whether the vasopressin release was obtained only from the caudally situated area which is indicated by the ovals B.

It has been shown (Guertzenstein, 1973) that three of the excitatory substances, tubocurarine, leptazol and strychnine, produce a strong rise in arterial blood pressure when applied to the more rostrally situated area, indicated by the ovals A, at the ventral surface. In the present experiments the effects of picrotoxin and bicuculline have been examined to see whether they have a similar action; in addition all five central excitatory substances have been applied to the more caudally situated area indicated by the ovals B and their effects on arterial blood pressure observed.

The central excitatory substances were applied to the ventral surface of the medulla through Perspex rings in small amounts but at high concentrations. The amount of solution placed in each ring was always 20 μl only, but the concentration of the different substances in the solution varied between 1 and 50 mg/ml. However, these high concentrations will not be those prevailing at the site of action because before the substances reach the nervous structures on which they act they have to pass the pia, a layer of glial fibres and perhaps a layer of nervous tissue as well. With each micron of penetration the concentration of the applied substances will decrease depending on their enzymatic inactivation, being affected also by their absorption into the blood capillaries and perhaps by being taken up and bound to nervous and glial structures. These factors have to be taken into account when evaluating physiologically the strong concentrations that are required for drugs to produce their effects on topical application to the ventral surface of the medulla.

Some of these results have been communicated to the British Pharmacological Society (Feldberg & Rocha e Silva Jr, 1977).

Methods

The experiments were carried out on cats weighing between 2.8 and 3.8 kg, anaesthetized by intravenous chloralose (0.65 mg/kg) after induction of anaesthesia with ethyl chloride and ether to allow cannulation of the left femoral vein. The trachea was cannulated, but unless otherwise stated, artificial ventilation was not applied. Arterial blood pressure was recorded from a cannula inserted into the right femoral artery by means of a transducer connected through a Cambridge pre-amplifier (Type 72342) to a Smith's Servoscribe potentiometric recorder. The cats were given an intravenous injection of atropine methyl nitrate which does not readily pass the blood-brain barrier.

The method used for applying the drugs topically to the exposed ventral surface of the brain stem through paired Perspex rings has been described in detail (Bisset *et al.*, 1975; Feldberg & Guertzenstein, 1976) and a diagram of the slightly oval rings with their holder has been published (Guertzenstein, 1973). The rings were placed across the medulla in either a more rostral or a more caudal position and the areas covered by the rings in these positions are indicated by the ovals A and B in the insets of Figures 6 and 7. The drugs were placed inside each ring in a volume of 20 μl and at the end of the experiments the areas covered by the drug solution were determined from the staining produced by filling the rings with 0.8% bromophenol solution according to the procedure described by Guertzenstein & Silver (1974).

Collection of the venous blood samples, their extraction and assay for vasopressin followed the procedures used by Bisset *et al.* (1975). The blood was collected from the inferior vena cava through a cannulated femoral vein. For each sample 5 ml of blood was withdrawn over a period of 2 min and replaced by the same amount of warmed dextran into the jugular vein.

The blood extracts were assayed for vasopressin by their antidiuretic activity on intravenous injection into water-loaded rats under ethanol anaesthesia, as described by Bisset, Clark & Errington (1971). The standard used for the assay was synthetic arginin vasopressin (Sandoz).

Drugs

The following drugs were used: nicotine hydrotartrate (BDH), tubocurarine chloride (Burroughs Wellcome & Co.), atropine methyl nitrate (Sigma) and strychnine hydrochloride (Hopkins Williams Ltd.) The amounts of these drugs given in the text refer to the salt except for nicotine when they refer to the base, in order to allow a direct comparison with the previous results obtained by Bisset *et al.* (1975). Leptazol (Boots), picrotoxin (Ralph N. Emanuel Ltd) and bicuculline (Pierce Chemical & Co.) were also used. The drugs were freshly dissolved in 0.9% w/v NaCl solution before use.

Results

Vasopressin release

All five central excitatory substances released vasopressin when applied bilaterally to the ventral surface of the medulla at the area indicated by the ovals B in the insets of Figures 6 and 7. The release developed gradually and showed no sign of tachyphylaxis.

Tubocurarine. In three experiments tubocurarine was applied in a concentration of 20 mg/ml. In the first experiment the vasopressin content of the plasma was 57 μu/ml in the blood sample collected before and 87 μu/ml in the sample collected during the third

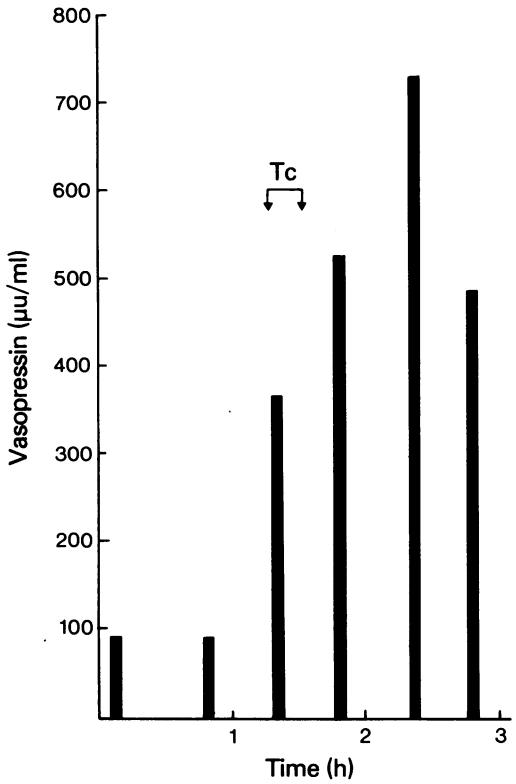

Figure 1 Vasopressin release in a cat anaesthetized with chloralose by tubocurarine (Tc) applied through paired Perspex rings to the ventral surface of the medulla in a concentration of 20 mg/ml for 16 minutes. In this and the following four figures, the black columns indicate the times when samples of venous blood were withdrawn and the height of the columns the vasopressin content of the plasma in μu/ml.

and fourth minute of the tubocurarine application. The tubocurarine was not washed out but remained in the rings until the next sample had been collected 40 min later; its vasopressin content had risen to 520 μu/ml. In the second experiment (Figure 1) the vasopressin content rose from 91 and 88 μu/ml in the samples collected before, to 367 μu/ml in the sample collected during the third and fourth minute of a 16 min application of tubocurarine. It continued to rise to 464 and 731 μu/ml in the two samples collected 30 and 60 min later, but then fell to 385 μu/ml at 90 minutes. With shorter application, the vasopressin also continued to rise after the application, but for a shorter period. In the third experiment, the tubocurarine was applied for 4 minutes. The highest vasopressin content was found in the sample collected 30 min after the application, but after 60 min the vasopressin had returned to the pre-application level.

Picrotoxin. Figure 2 illustrates the vasopressin release in two experiments in which picrotoxin was applied in a concentration of 2 mg/ml. In one experiment (Figure 2a) the picrotoxin was applied for 26 minutes. There was a small rise of vasopressin in the sample collected during the third and fourth minute of application, from 106 to 145 μu/ml, but the vasopressin continued to rise and reached a value of 381 μu/ml in the sample collected 5–6 min after the application. It then came down first to 160 and then to 85 μu/ml in the following two samples, each collected after an interval of about half an hour. On subsequent application of nicotine (4 mg/ml) the vasopressin rose again, this time to 253 μu/ml in the sample collected during the second and third minute of application.

In the other experiment (Figure 2b) the picrotoxin was applied for 6 minutes. There was a small rise, from 23 to 32 μu/ml in the sample collected during the third and fourth minute of application. Again the vasopressin continued to rise, this time to 135 and then to 225 μu/ml in the samples collected about 30 and 60 min later; it then fell to 145 μu/ml after about another 30 minutes. Two minutes later, nicotine (4 mg/ml) was applied; this resulted in a renewed rise, this time to 415 μu/ml.

Bicuculline. In two experiments bicuculline was applied in a concentration of 2 mg/ml for about 20 minutes. The result was practically the same. In both there was release of vasopressin which continued and increased for some time after the application and nicotine (4 mg/ml), applied after the effect of bicuculline had come to an end, produced a release greater than that obtained with bicuculline. One of the experiments is illustrated in Figure 3.

Leptazol. The vasopressin release by this weak central excitatory substance was of shorter duration than that produced by tubocurarine, picrotoxin, or bicuculline, in that it diminished as soon as the leptazol was washed out of the rings. On the other hand, release continued as long as the leptazol (50 mg/ml) was left in place (Figure 4).

Strychnine. In one experiment strychnine was applied in a concentration of 10 mg/ml for 5 min, in another in a concentration of 5 mg/ml for 19 minutes. In both the vasopressin release resembled the release produced by tubocurarine, picrotoxin and bicuculline, developing more gradually and being of longer duration than the release produced by leptazol (Figure 5). Later nicotine (4 mg/ml) produced a release which was greater in the experiment (Figure

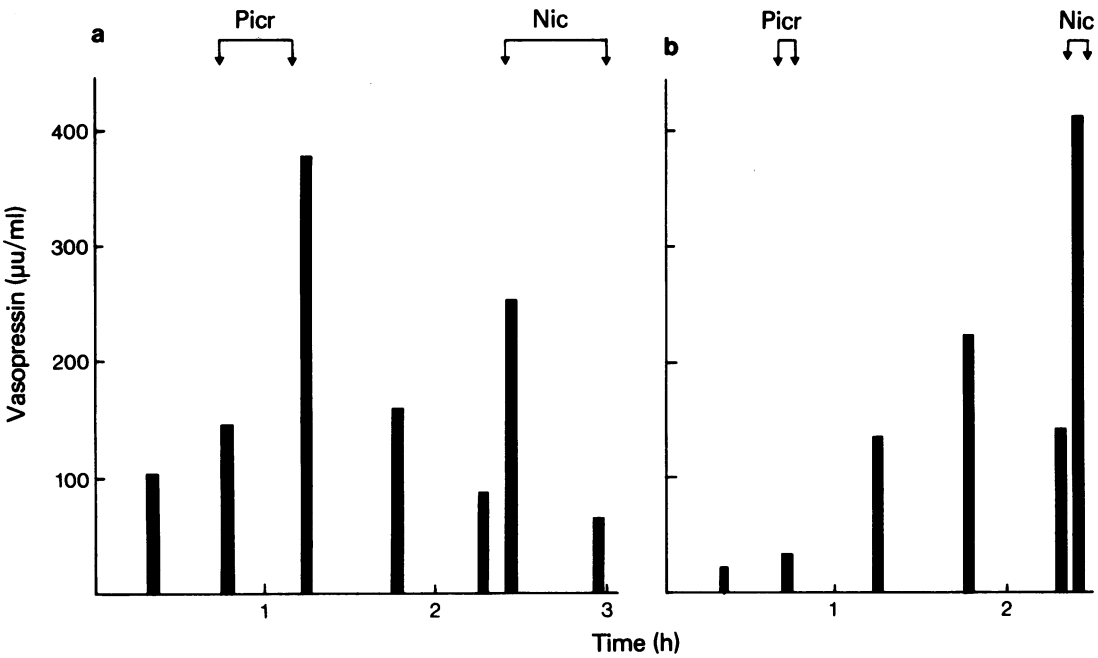

Figure 2 Vasopressin release in two cats anaesthetized with chloralose by picrotoxin (Picr) and nicotine (Nic) applied through paired Perspex rings to the ventral surface of the medulla; picrotoxin was applied in a concentration of 2 mg/ml for 26 min in experiment (a) and for 6 min in experiment (b). Nicotine was applied in a concentration of 4 mg/ml for 36 min in (a) and for 6 min (b).

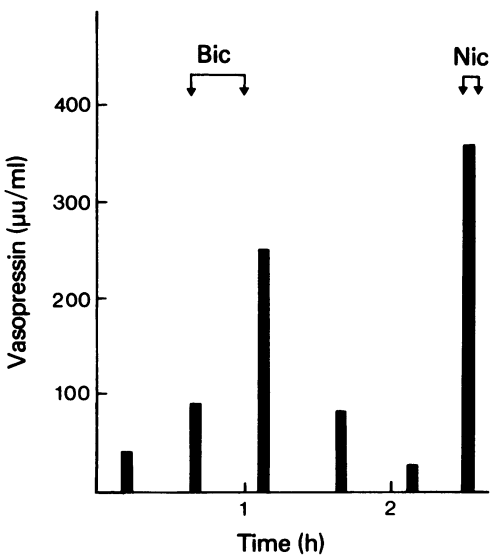

Figure 3 Vasopressin release in a cat anaesthetized with chloralose by bicuculline (Bic) and by nicotine (Nic) applied through paired Perspex rings to the ventral surface of the medulla, bicuculline in a concentration of 2 mg/ml for 20 min and nicotine in a concentration of 4 mg/ml for 6 minutes.

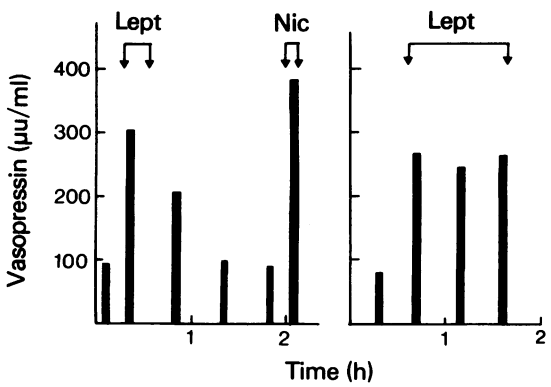

Figure 4 Vasopressin release in two cats anaesthetized with chloralose by leptazol (Lept) and nicotine (Nic) applied through paired Perspex rings to the ventral surface of the medulla. In both experiments, leptazol was applied in a concentration of 50 mg/ml, in experiment (a) for 16 min, and nicotine in a concentration of 4 mg/ml for 4 minutes. In experiment (b), leptazol was applied for 62 minutes.

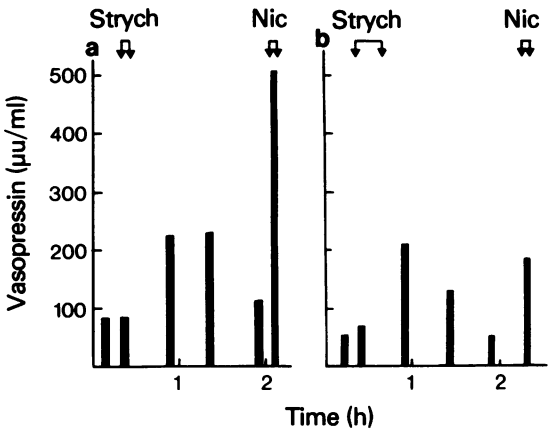

Figure 5 Vasopressin release in two cats anaesthetized with chloralose by strychnine (Strych) and nicotine (Nic) applied through paired Perspex rings to the ventral surface of the medulla. In experiment (a), strychnine was applied in a concentration of 10 mg/ml for 5 min, and in (b) in a concentration of 5 mg/ml for 19 minutes. In both experiments nicotine (4 mg/ml) was applied for 6 minutes.

5a) in which it was applied before the strychnine effect had subsided.

Blood pressure effects

All five central excitatory substances produced strong pressor responses when applied to the rostral area at the ventral surface of the brain stem, indicated by the ovals A, in the insets of Figures 6 and 7, but when applied to the caudal area, indicated by the ovals B, they had either no effect on blood pressure, or produced weak pressor responses only. The time it took for the pressor responses to develop and to subside was longest with tubocurarine and shortest with leptazol. The results obtained with picrotoxin, bicuculline and leptazol are illustrated in Figures 6 and 7.

Guertzenstein had already found in 1973 that the strong pressor responses produced by tubocurarine applied to the rostral area developed and subsided much more gradually than those produced by leptazol and strychnine, similarly applied. In the present experiments it was found that the strong pressor responses obtained with picrotoxin from area A also developed and subsided gradually but not as gradually as with tubocurarine. Those produced by bicuculline developed and declined more rapidly than those produced by picrotoxin and those produced by leptazol and strychnine developed and subsided rapidly.

Discussion

With the finding that tubocurarine, picrotoxin, bicuculline, leptazol and strychnine release vasopressin when applied to the ventral surface of the brain stem, a new pharmacological property has been brought to light for these central excitatory substances. Four of them were shown to be antagonists of γ-aminobutyric acid (GABA) and/or of glycine. Tubocurarine was shown to be an antagonist not only of GABA (Hill, Simmonds & Straughan, 1972; 1973) but also of glycine (Curtis, Game & McCulloch, 1974), picrotoxin and bicuculline to be antagonists of GABA (Curtis, Duggan, Felix & Johnston, 1970; Hill *et al.* 1973), and strychnine has long been known to be an antagonist of glycine (for references see Curtis & Johnston, 1974). No evidence has yet been found that leptazol is an antagonist of GABA, but it is such a weak convulsant that, as pointed out by Hill *et al.* (1973), 'a sufficient concentration of leptazol to produce GABA antagonism may not be achieved in the area of the neurone when the drug is applied by iontophoresis', the method commonly used for quantitative studies of the antagonism.

It may therefore well be that all five central excitatory substances release vasopressin by antagonizing the action of GABA and/or of glycine. This would imply that vasopressin release in the body is continuously inhibited by the activity of GABA and glycine releasing neurones which synapse at or near the ventral surface of the brain stem, and from the large increases in vasopressin release obtained with the antagonists of GABA and glycine it would follow that this inhibition is an extremely powerful one.

It would be interesting to know if nervous or other influences which release vasopressin do so by removing the 'amino acid brake' and if so, whether increasing the brake by applying GABA and glycine to the ventral surface would prevent or attenuate the release produced by such influences.

The vasopressin is released either from the supraoptic nuclei alone or from the paraventricular nuclei as well, but nothing is known about the afferent nervous pathway from the medulla to these nuclei. If the vasopressin release were continuously inhibited by the activity of GABA and glycine releasing neurones which synapse at the ventral surface, interruption of the afferent nervous pathway might reveal itself by increased vasopressin release.

The vasopressin release produced by the five central excitatory substances differed from that produced by nicotine which suggests a different mode of action. With the central excitatory substances the release proceeded gradually and showed no sign of tachyphylaxis. With nicotine it occurred almost instantaneously and then stopped because tachyphylaxis developed. This tachyphylaxis resembled the action of

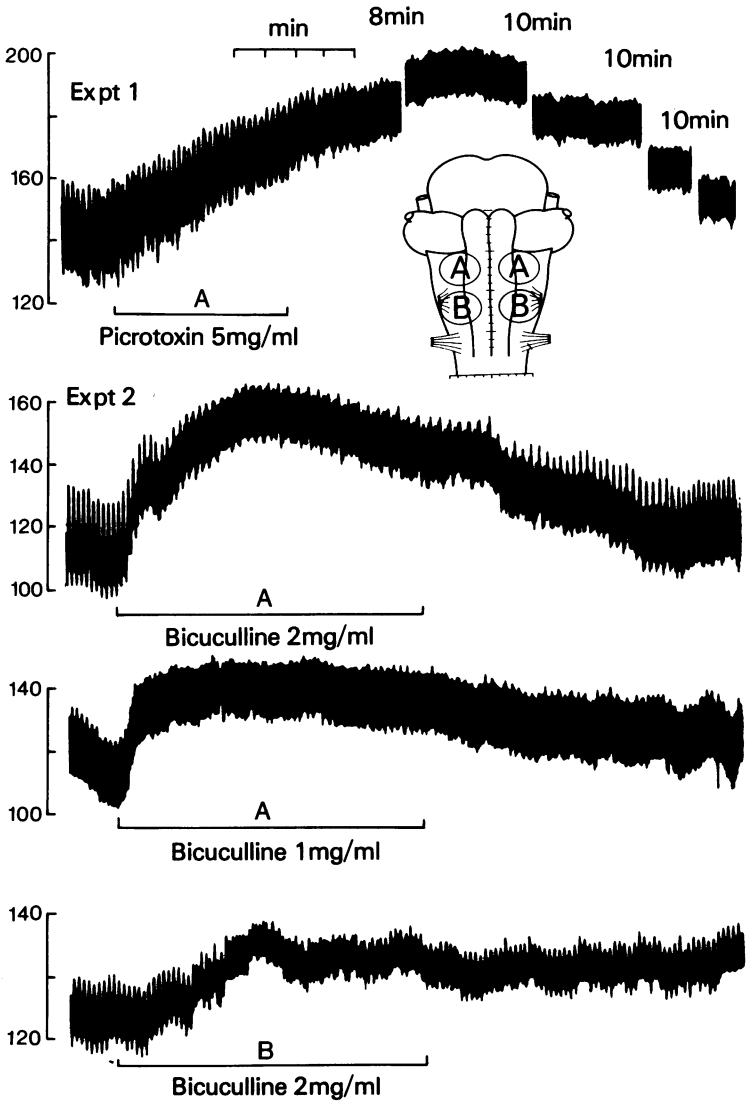

Figure 6 Records of arterial blood pressure from two cats anaesthetized with chloralose. Effect of application through paired Perspex rings to the ventral surface of the medulla of picrotoxin and bicuculline in the concentrations given beneath the horizontal lines which indicate the duration of the applications. The letters A and B above the lines refer to the areas covered by the Perspex rings, indicated in the inset by the similarly marked ovals.

nicotine on automatic ganglia; they, too, are first stimulated and then rendered insensitive to nicotine. The ganglion blocking agent, hexamethonium, shared with nicotine the ability to block the release of vasopressin (Bisset & Feldberg, 1977).

The site where the central excitatory substances act at the ventral surface when they release vasopressin may not be exactly the same site at which nicotine acts because the methods used for applying these drugs through paired Perspex rings would be too crude to detect small differences. If they act at the same site they need not necessarily act at the same postsynaptic neurones, although there is no reason to assume an action at different neurones, even if it

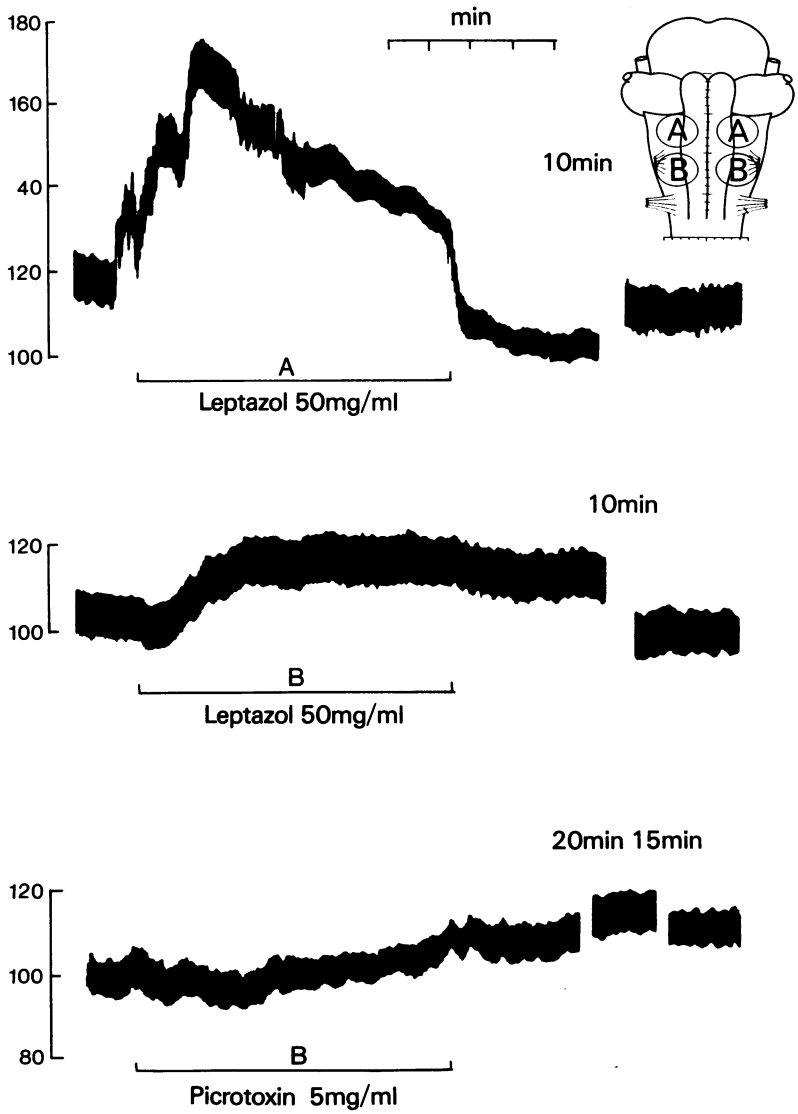

Figure 7 Records of arterial blood pressure from a cat anaesthetized with chloralose. Effect of application through paired Perspex rings to the ventral surface of the medulla of leptazol and picrotoxin in the concentrations given beneath the horizontal lines, which indicate the duration of the applications. Inset as in Figure 6.

should be shown that the vasopressin release produced by the central excitatory substances were not prevented by persistence of nicotine or by hexamethonium. A nerve cell may become insensitive to one drug but retain its sensitivity to another.

There is a striking difference between the blood pressure effects produced by nicotine and by the central excitatory substances, not only when applied to the nicotine-sensitive area but also when applied to the more rostrally situated area indicated by the ovals A in Figures 6 and 7. The effect of nicotine was a fall in arterial blood pressure, obtained only from the more caudally situated area. The effect of the central excitatory substances was a rise in arterial blood pressure, but strong pressor responses were obtained only from the more rostrally situated area. Applied to the

more caudally situated area they had either no effect on blood pressure, or produced merely weak pressor responses.

The pressor responses of the central excitatory substances could result from antagonizing the action of GABA and glycine continuously released from inhibitory neurones which synapse at or near the ventral surface of the medulla. This mode of action had been suggested by Guertzenstein (1973) when he found that, applied to the more rostrally situated area of the ventral surface, tubocurarine, leptazol and strychnine produced the opposite effect on blood pressure, a strong pressor response, from GABA and glycine which produced strong depressor responses. At the more distally situated nicotine-sensitive area few, if any, such inhibitory neurones associated with blood pressure reactions appear to synapse, because, applied to this area, GABA and glycine were found either not to affect blood pressure, or to produce a fall or a rise of not more than 10 mmHg (Feldberg & Guertzenstein, 1976). It may be that numerous GABA and glycine releasing inhibitory neurones end at both areas of the ventral surface, but that those ending at the more rostrally situated area are concerned with blood pressure reactions, those ending at the more caudally situated nicotine-sensitive area, with inhibition of vasopressin release.

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