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VASOPRESSIN RELEASE BY NICOTINE: THE SITE OF ACTION

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1 In cats anaesthetized with chloralose the release of neurohypophysial hormones was examined after injection of nicotine into the cerebral ventricles or cisterna magna or its topical application through perspex rings to the ventral surface of the brain stem. The release was measured by assaying the hormones in samples of venous blood

2 Injected into a lateral or the third cerebral ventricle, nicotine (0.5 to 1 mg) produced release of vasopressin without oxytocin. When the aqueduct was cannulated, preventing access to the fourth ventricle and to the subarachnoid space, this release did not occur.

3 Vasopressin was also released without oxytocin when nicotine (0.25 to 2 mg) was injected into the subarachnoid space through the cisterna magna. With this route of administration the nicotine did not enter any part of the ventricular system.

4 Applied through paired perspex rings placed across the ventral surface of the brain stem, nicotine again produced release of vasopressin without oxytocin. The amount of nicotine placed in each ring was usually 80 μg, but a release was obtained with 10 μg and in one experiment with as little as 5 μg.

5 The bilateral region on the ventral surface of the brain stem where nicotine acts when producing release of vasopressin lies lateral to the pyramids and in a longitudinal direction, 6 to 9 mm caudal to the trapezoid bodies.

6 The vasopressin release by nicotine injected intraventricularly or intracisternally, or applied topically to the ventral surface of the brain stem was not due to absorption of nicotine into the blood stream, nor to blood pressure effects.

7 It is concluded that nicotine acts on the ventral surface of the brain stem probably by activating the central projection to the supra-optic and possibly also the paraventricular nuclei of afferent pathways in the sinus and vagus nerves which control the release of vasopressin in response to changes in blood volume or distribution.

Introduction

It is generally agreed that the paraventricular nuclei (PVN) are involved in the release of oxytocin, but whether these nuclei also release vasopressin is controversial. The PVN contain oxytocin and vasopressin in all species examined. Yet in the lactating guinea-pig (Tindal, Knaggs & Turvey, 1968), and rabbit (Aulsebrook & Holland, 1969), their electrical stimulation causes milk ejection without a rise in arterial blood pressure, suggesting release of oxytocin alone. On the other hand, in the cat, electrical stimulation of the PVN increases the concentration of both hormones in the blood (Bisset, Clark & Errington, 1971).

The PVN should be readily accessible to drugs injected into the cerebral ventricles, as they are contiguous to the walls of the third ventricle. The possibility was therefore considered of stimulating these nuclei by a drug injected into the cerebral ventricles in order to find out if oxytocin alone, or oxytocin with vasopressin would be released. Nicotine was chosen for this purpose. It is the drug which has been most widely used for studying the release of neurohypophysial hormones since the discovery of its antidiuretic action by Burn, Truelove & Burn (1945).

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However, when nicotine was injected into the cerebral ventricles of the anaesthetized cat, it was found to increase the concentration of vasopressin in the blood, but not of oxytocin. This unexpected finding made it doubtful that its action was on the PVN, or that it acted at all on structures lining the ventricular walls.

Cats have no foramen of Magendie, which exists only in primates, and drugs injected into the cerebral ventricles of cats pass rapidly into the subarachnoid space through the lateral recesses of the fourth ventricle and reach the ventral surface of the brain stem. Recently, it has been shown that a number of drugs which, on intraventricular injection affect blood pressure, heart rate, respiration or blood glucose, act in this way (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973; Bousquet & Guertzenstein, 1973; Edery & Guertzenstein, 1974; Feldberg & Gupta, 1974; Guertzenstein & Silver, 1974; Dey, Feldberg & Wendlandt, 1975).

The following procedures are used to determine if the ventral surface of the brain stem is the site at which a drug acts on intraventricular injection. First, it is injected into a lateral or the third ventricle after cannulation of the cerebral aqueduct. If its effect is abolished the drug cannot act on structures lining these ventricles. It is then injected into the subarachnoid space through the cisterna magna. If this is effective, the likely site is either on the ventral or dorsal surface of the brain stem, since there is no access to the ventricles from the cisterna. Finally, the site can be localized by applying the drug topically through perspex rings to these surfaces. All three procedures were used in the present experiments to determine the site where nicotine acts. The technique of topical application was employed on the ventral surface only since this surface is reached more readily than the dorsal one on intraventricular injection.

Some of the results have been communicated to the Physiological Society (Bisset & Feldberg, 1972).

Methods

The experiments were done on anaesthetized male and female cats weighing between 3 and 4.5 kg. Some of the female cats were lactating. Anaesthesia was induced with ethyl chloride and ether followed by intravenous chloralose (70 mg/kg). The trachea was cannulated and the head of the cat was fixed to the ear bars and mouthpiece of a stereotaxic instrument. In the experiments in which the nicotine was injected into the cerebral ventricles or into the cisterna magna, the cat was lying on its belly; in those in which the nicotine was applied to the exposed ventral surface of the brain stem the cat was put on its back. Blood pressure was recorded from a femoral artery with a Statham strain gauge transducer and a Goertz potentiometric recorder (1 mmHg = 1.33 mbar). The same method of recording was used for determining the milk-ejection pressure from a cannulated teat duct in lactating cats in order to monitor the release of neurohypophysial hormones as described by Bisset, *et al.* (1971).

In order to prevent inhibition of the milk-ejection response by any adrenaline or noradrenaline that might be released into the circulation by nicotine, oxprenolol hydrochloride (Trasicor, Ciba) (4 mg) was injected intravenously because this β-adrenoceptor blocking agent has been shown to prevent inhibition of the milk-ejection response produced by adrenaline (Bisset, Clark & Lewis, 1967; Bisset *et al.*, 1971). Oxprenolol was given routinely also to the non-lactating cats to maintain the same experimental conditions. Atropine sulphate (4 mg) was routinely injected intravenously to prevent hypotension produced by release of acetylcholine from cholinergic neurones; in preliminary experiments it had been found not to prevent the release of vasopressin on intraventricular injection of nicotine.

The methods used for cannulating a lateral or the third cerebral ventricle with a Collison cannula were those described by Feldberg & Shaligram (1972). To cannulate the aqueduct a polythene tube of about 5 cm length was inserted through the opened cisterna along the floor of the fourth ventricle into the middle of the aqueduct so as to prevent any ventricular fluid from passing into the fourth ventricle. The outer end of the tube was bent to take up a nearly vertical position. This ensured that at least part of the nicotine solution (0.15 ml) injected into the cerebral ventricle stayed in the ventricles for some time. Injections into the cisterna magna were made as described by Feldberg & Guertzenstein (1972).

The method of exposing the ventral surface of the brain stem was that described by Feldberg & Guertzenstein (1972) except when the nicotine was applied to regions caudal to the rootlets of the XIIth cranial nerves. A diagram of the paired rings with their holder through which the nicotine was applied has been published (Guertzenstein, 1973). The rings were slightly oval, their inner diameters being 5 and 4 mm, and the distance between them 2 mm. They were placed across the medulla, each at the same distance from the midline. In order to be able to apply the nicotine to regions caudal to the rootlets of the XIIth cranial nerves, the ventral arch of the atlas as well

as the dens of the epistropheus was removed with nibbling forceps and the tough dura containing a transverse venous sinus was tied on both sides as far from the midline as possible. By cutting the dura between the ligatures the ventral surface was exposed sufficiently to place the perspex rings so as to cover the regions of the rootlets of the 1st cervical nerves. The nicotine was applied in a volume of 20 μl in each ring and washed out immediately after a blood sample had been collected. The nicotine salt used was the hydrogen-tartrate but all values given in the text refer to the base. In the concentrations used the nicotine solutions were strongly acid but the vasopressin release was not due to the acidity as the release was also obtained when the solutions were neutralized. For instance, the largest vasopressin release on topical application was obtained in an experiment (No. 9 of Table 2) in which the pH of the nicotine solution was adjusted to pH 7.2.

To determine the areas on the ventral surface which were covered by the nicotine solution placed in the rings, the procedure described by Guertzenstein & Silver (1974) was adopted. The rings were filled with a 0.8% bromophenol blue solution; 5-10 min later the cat was killed by an overdose of intravenous pentobarbitone sodium. Before the rings were removed they were washed out several times with 0.9% w/v NaCl solution (saline) and the stained areas on the ventral surface were measured with a divider, using as references the caudal border of the trapezoid bodies and the mid line. The circles shown in the diagrams of Figures 3 and 4 were obtained in this way. In drawing the circles, slight variations in transverse direction were not taken into consideration but the rostral and caudal borders which were about 4 mm apart were determined within 0.5 mm.

Collection of blood samples

Samples of blood coming from the head were obtained from a cannula inserted upwards into the external jugular vein. Each sample (5 ml) was withdrawn into a nylon syringe containing a trace of heparin at a steady rate over 2 min with simultaneous replacement of warm dextran solution into the inferior vena cava through a cannulated femoral vein. In some experiments in which difficulty was experienced in withdrawing blood, the sample was taken from the femoral vein and dextran replaced through the external jugular vein.

The results obtained with the two procedures were the same.

Extraction and assay of blood samples

The samples were extracted by the method of Bisset, Hilton & Poisner (1967) which consists of precipitating the plasma proteins with alcohol and concentrating the supernatant. The extracts were assayed for vasopressin by their antidiuretic activity on intravenous injection into water-loaded rats under ethanol anaesthesia, and for oxytocin by their milk-ejecting activity on retrograde arterial injection into lactating rats treated with oxprenolol. These assay methods have been described in detail by Bisset *et al.* (1971). The standards used for the assay were synthetic arginine-vasopressin (Sandoz) and synthetic oxytocin (Syntocinon, Sandoz). The potency of the arginine-vasopressin was confirmed by assaying its antidiuretic activity against that of the III International Standard for Posterior Pituitary (Bangham & Musset, 1958).

In the assay for oxytocin, interference from the intrinsic milk-ejecting activity of vasopressin (Bisset *et al.*, 1971) was avoided by treating the extracts with trypsin before assaying them for milk-ejecting activity. Trypsin is known to inactivate vasopressin in brain extracts without affecting oxytocin (Bisset, Errington & Richards, 1973). The procedure used for blood extracts was to add 100 μg of trypsin (in 0.1 ml) to 0.5 ml extract and to incubate the mixture at 38°C for 60 min at pH 7.5. This resulted in a loss of 95% of the antidiuretic activity in the extracts. On the other hand, when a known amount of oxytocin was added to a blood sample before extraction the trypsin treatment did not reduce the recovery of its milk-ejecting activity which amounted to 95%.

In every experiment in which no oxytocin was detected in an extract, that extract was re-tested after the addition of a threshold amount of oxytocin to find out if it contained an inhibitory factor which might have masked the presence of oxytocin. The response to the added oxytocin was always found to be uninhibited.

The identity of the vasopressin in the extracts was established by showing that their antidiuretic activity was greatly reduced or abolished by treatment with trypsin, with sodium thioglycollate (Bisset *et al.*, 1971) and with a specific antibody to vasopressin (Bisset, Black, Hilton, Jones, Kanjanapothi & Montgomery, 1974).

Results

Intraventricular and intracisternal injections

As shown in Table 1, injections of 1 mg of nicotine into the cerebral ventricles caused the

release of vasopressin into the blood without a release of oxytocin whether the injections were made into the third (cats 1 to 4) or into a lateral ventricle (cats 5 and 6). The vasopressin increased between 6 and 32-fold in blood samples withdrawn over a period of 2 min beginning 35 s after the injection, but in samples collected 20 min later, the vasopressin content was again low and had sometimes returned to the pre-injection level.

The collection of the blood samples was begun 35 s after the injection because in two experiments (Expts 1 and 4) on lactating cats with a teat duct cannulated, the milk-ejection pressure began to rise steeply about 35 s after the injection of 1 mg nicotine, and the rise was shown to be an effect not of released oxytocin but of released vasopressin. This is illustrated in Figure 1 which is from the same cat as Expt 1 of Table 1. The records show the effects of intravenous injections of oxytocin and of intravenous and intraventricular injections of nicotine on the milk-ejection pressure. An intravenous injection of 4 mu of oxytocin produced a large rise in pressure (at (a) in Figure 1) whereas 2 mu had a just perceptible effect (at (b)). The intraventricular injection of 0.1 mg nicotine (at (c)) had no effect on milk-ejection pressure, nor did it increase the oxytocin or vasopressin content of the blood. However, 35 s after the intraventricular injection of 1 mg nicotine (at (d)) the milk-ejection pressure began to rise steeply and the rise was of the same magnitude as that produced by 4 mu oxytocin. Yet, in the blood sample, collection of which began the moment the pressure rose, the oxytocin content was not increased, whereas its vasopressin content had risen from < 13 to 630 μu/ml. A similar result was obtained when the intraventricular injection of 1 mg nicotine was repeated after 70 min (at (e)).

Figure 1 further illustrates that absorption of nicotine into the blood stream was not responsible for the rise in milk-ejection pressure. Even if half of the 1 mg were absorbed within 35 s, although any absorption which occurs from the liquor space is far too slow for such a possibility to be contemplated, this would not have been sufficient

Table 1 Vasopressin (V) and oxytocin (O) in blood collected before (I), 35-155 s (II), and 20 min (III) after injection of nicotine into the third ventricle (cats 1-4), into a lateral ventricle (cats 5 and 6), and into the cisterna magna (cats 7 and 8)

		μu/ml of V or O					
		I		II		III	
Cat no.	*Nicotine (mg)*	*V*	*O*	*V*	*O*	*V*	*O*
1	0.02	22	<22	17	<11		
	0.1	22	<22	<13	<26		
	1			630	<13		
	1			560	<12		
2	1	<10	10-20	266	<10	15	10-20
3	1	16	10	152	<16	96	10
	1*	30	<16	660	20	216	10
4	1*	<10	<20	60	<8	<10	20
5	1	<30	<39	960	<39	97	<39
	1	<30	<39	476	<44	72	<47
	1≠	<30	<39	50	<65		
6	1	29	<48	504	<40	60	40
	1≠	42	<40	80	<80		
	1φ			303	<40	165	<40
7	2	25	<33	900	<33		
	0.5	24	<33	156	<33	92	<33
	2			390	<33		
8	0.5	48	<22	395	<22	48	<22
	0.25	<20	<22	216	<22	39	<22
	0.125			<20	<22		

Cats 1 and 4 lactating. * Carotid sinus denervated and vagi cut. ≠ Aqueduct cannulated. φ Tip of aqueductal cannula withdrawn to caudal end of fourth ventricle.

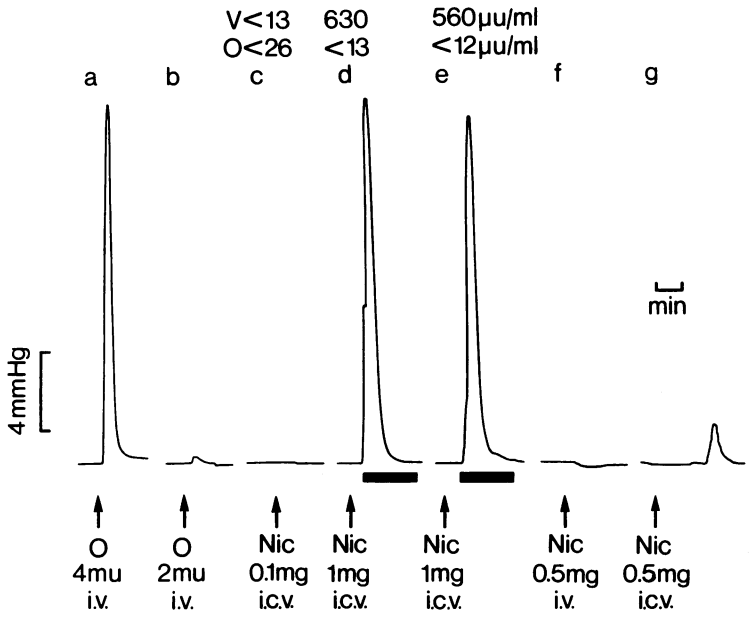

Figure 1 Records of milk-ejection pressure from a cannulated teat duct of a lactating cat anaesthetized with intravenous chloralose. Same experiment as No. 1, Table 1. Responses to intravenous (i.v.) injections of 2 and 4 mu oxytocin (O) and of 0.5 mg nicotine (Nic) and to intraventricular (i.c.v.) injections of 0.1, 0.5 and 1 mg nicotine. The figures on top give vasopressin (V) and oxytocin (O) content in μu/ml in the 2 ml samples of blood collected 35 to 155 s after the injections of 0.1 and 1 mg nicotine. The 2 min period of collection is indicated for the injections of 1 mg nicotine below the records by a solid bar.

to account for the rise in milk-ejection pressure because 0.5 mg nicotine injected intravenously (at (f)) had no effect; injected intraventricularly (at (g)), however, it was sufficient to bring about a small delayed response.

The intraventricular injection of 1 mg of nicotine produced changes in arterial blood pressure. The more usual response was a large rise in blood pressure but sometimes the response consisted mainly of a fall. It is known that a fall in blood pressure, provided it is sufficiently large and steep, causes release of vasopressin without release of oxytocin (Beleslin, Bisset, Haldar & Polak, 1967). However, the depressor effect when produced by an intraventricular injection of 1 mg of nicotine was insufficient to do so because much smaller doses of nicotine, which were ineffective in releasing vasopressin, produced larger depressor effects. This is illustrated in Figure 2 which is from the same experiment as Figure 1. Further evidence for the conclusion that the vasopressin release was independent of the fall in blood pressure was obtained in experiments in which the carotid sinuses were denervated and the vagi cut in the neck. These procedures, which are known to abolish the vasopressin release associated with the hypotension during haemorrhage (Clark & Rocha e Silva Jr, 1967) or produced by hypotensive drugs (Bisset, Kanjanapothi & Rocha e Silva Jr, unpublished experiments) did not abolish the vasopressin release produced by an intraventricular injection of 1 mg of nicotine. Experiment 4 of Table 1, shows the release produced by the nicotine injection after, and Expt 3 before and after denervating the carotid sinuses and cutting the vagi.

Two series of experiments suggested that the action of nicotine in releasing vasopressin was not on structures lining the ventricular cavities, but on structures reached after the nicotine had passed into the subarachnoid space. First, hardly any release of vasopressin occurred on intraventricular injection of 1 mg of nicotine when the aqueduct was cannulated and the passage of nicotine into the subarachnoid space was prevented. Secondly, a release occurred when the nicotine was injected into the cisterna magna, from which there is no access to the ventricles.

The effect of cannulation of the aqueduct is seen in Expts 5 and 6 of Table 1. In Expt 5, this was done after the first two intraventricular injections of 1 mg nicotine which increased the

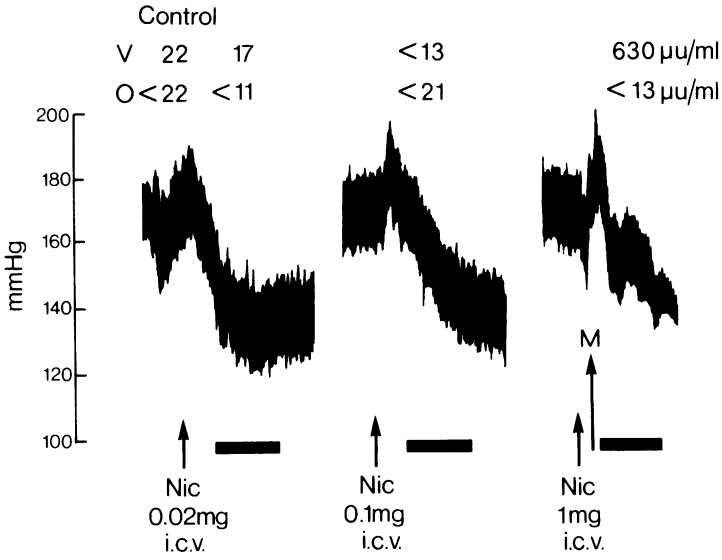

Figure 2 Arterial blood pressure from a lactating cat anaesthetized with intravenous chloralose. Same experiment as that of Figure 1. At the arrows marked Nic, intraventricular (i.c.v.) injections of 0.02, 0.1 and 1 mg nicotine. The arrow marked M indicates beginning of rise in milk-ejection pressure. The 2 min periods of collection of the blood samples are indicated below the records by solid bars. The figures on top refer to vasopressin (V) and oxytocin (O) content in μu/ml of the collected samples.

vasopressin in the blood from < 30 to 960 μu/ml after the first, and to 476 μu/ml after the second injection. The third intraventricular injection of 1 mg nicotine which was done with the aqueduct cannulated, caused a rise to 50 μu/ml only. In Expt 6, the aqueduct was cannulated after the first injection of 1 mg nicotine which had increased the vasopressin in the blood from < 48 to 504 μu/ml, but the second injection with the aqueduct cannulated caused an increase to 80 μu/ml only. The cannula was then withdrawn to the caudal end of the fourth ventricle to re-open the passage of c.s.f. through the lateral recesses and the flow into the subarachnoid space was facilitated by keeping the free end of the cannula a little above the head. In this condition, the injection of 1 mg nicotine produced again a large increase in the vasopressin content of the blood which rose to 303 μu/ml.

Both in Expts 5 and 6, the cannulation of the aqueduct had been effective in preventing the nicotine from entering the subarachnoid space. This was evident because the injection was not followed by ear twitching. Without cannulation of the aqueduct, or when the tip of the aqueductal cannula was withdrawn to the caudal end of the fourth ventricle, the injection of nicotine produced intense ear twitching. This is due to an action on structures close to the dorsal surface of the upper cervical cord which are reached by the nicotine after having entered the subarachnoid space through the lateral recesses (Armitage, Milton & Morrison, 1966; Hall & Reit, 1966). In one experiment not included in the Table, in which the tip of the aqueductal cannula did not fit the walls of the aqueduct closely and some of the injected nicotine leaked into the fourth ventricle and then passed into the subarachnoid space, ear twitching occurred and the vasopressin content of the blood rose after the injection although not as high as without cannulation.

Experiments 7 and 8 of Table 1, show the effectiveness of nicotine in releasing vasopressin on injection into the cisterna magna. Doses between 0.25 and 2 mg produced a large increase of vasopressin in the blood without any increase of oxytocin. The injections also produced intense ear twitching. As shown in Expt 8, a dose of 0.125 mg was below threshold for vasopressin release.

Topical application

The release of vasopressin without oxytocin obtained on injection of nicotine into the cerebral ventricles or into the cisterna magna was reproduced by applying nicotine bilaterally, by means of perspex rings to the caudal part of the ventral surface of the medulla oblongata. A typical experiment is illustrated in Figure 3.

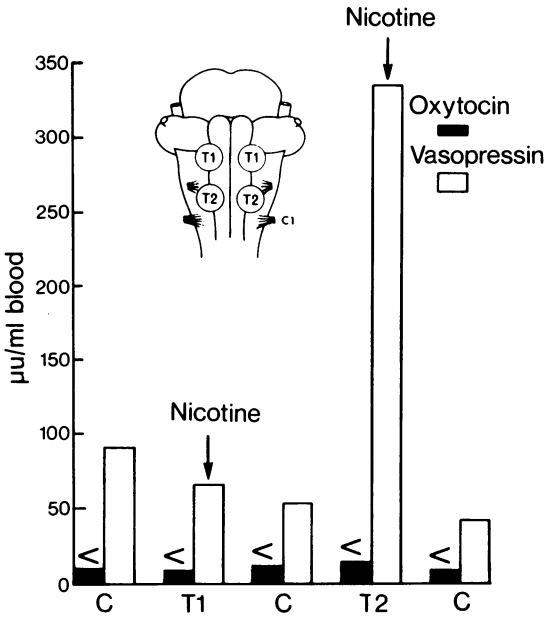

Figure 3 Oxytocin and vasopressin release on bilateral topical application of nicotine by means of perspex rings (80 μg in each ring) to two different regions of the ventral surface of the brain stem of cat. The two regions are indicated by the circles T_1 and T_2 in the diagram. C_1 rootlets of 1st cervical nerves; the rootlets above are those of the XIIth cranial nerves. Same experiment as No. 2 and No. 8 of Table 2. (For details see text.)

In this experiment, the nicotine was applied to two different regions. The regions on the ventral surface enclosed by the perspex rings during the first application of nicotine are indicated in the diagram of Figure 3 by the circles marked T_1. The regions overlap rostrally the caudal border of the trapezoid bodies by about 1 mm. The application of nicotine in the rings caused no release of oxytocin or vasopressin. A control blood sample (C) collected 20 min before the nicotine application contained < 7 μu/ml oxytocin and 89 μu/ml vasopressin and a sample, collection of which began about 35 s after placing 80 μg nicotine in each ring, contained < 6 μu/ml oxytocin and 65 μu/ml vasopressin. The nicotine was then washed out, and the rings were placed more caudally. Their new position is indicated in the diagram by the circles marked T_2; the most rostral points of the regions enclosed by the perspex rings now lay about 5 mm caudal to the trapezoid bodies and the relation of the regions to the rootlets of the XIIth cranial and the Ist cervical nerves is indicated in the diagram. From this region, nicotine produced release of vasopressin without oxytocin. Before placing the nicotine in the rings, about 1 h after collection of the second blood sample, a new control sample was collected; it contained even less vasopressin (52 μu/ml) than the first control and the oxytocin was still below threshold, < 11 μu/ml. The subsequent sample was collected a few minutes later; its collection began about 35 s after placing 80 μg nicotine in each ring. The vasopressin content of this sample increased to 334 μu/ml whereas the oxytocin content remained low, < 13 μu/ml. The nicotine was then washed out and about an hour later a further control sample of blood was withdrawn. Its vasopressin content was again low, 41 μu/ml, and its oxytocin content was < 8 μu/ml.

To determine the rostral and caudal border of the bilateral region from which vasopressin release could be obtained with nicotine, its effect was examined in different cats by placing the perspex rings at different distances caudal to the trapezoid bodies and examining the efficacy of nicotine when placing it in the rings, and sometimes also when spreading it in the same amount outside the rings either just rostral or caudal to them.

The results of 13 experiments (Nos 1-13) obtained on 12 cats are summarized in Table 2 and the regions on the ventral surface enclosed by the rings are shown by the circles in the diagram of Figure 4. No vasopressin release was obtained with nicotine placed in the rings when the rostral border of the regions enclosed by the rings either overlapped the trapezoid bodies or lay 1.5 to 2 mm caudal to them (Expts 1-4) and again when the rostral border lay 9 mm caudal to the trapezoid bodies (Expt 13). However, when the rostral border lay between 2.5 and 6 mm and the caudal border between 6.5 and 10 mm caudal to the trapezoid bodies (Expts 5-12), there was always a large release of vasopressin when the nicotine was placed in the rings. This would confine the nicotine sensitive area in longitudinal direction to between 6 and 9 mm caudal to the trapezoid bodies. This is also evident from the diagram of Figure 4. The dotted circles on the left side give the position of the rings from which no release, and the continuous circles on the right side the position of the rings from where a release of vasopressin was obtained with nicotine placed inside the rings. The same localization was obtained in those experiments in which the nicotine was spread onto the ventral surface either just caudal or rostral to the rings. In Expts 5, 7, 10 and 12, in which nicotine placed in the rings caused a release of vasopressin, no release occurred when the nicotine was spread just rostral to the rings, and in Expts 1 and 3, in which the rings were placed too far rostrally for nicotine to be

Table 2 Effect on arterial blood pressure and vasopressin content of blood, of topical application of nicotine to different regions of the ventral surface of the brain stem of cat

Expt no.	*Distance (mm) from trapezoid bodies of rostral (R) and caudal (C) border of regions enclosed by rings* R	C	*Changes in arterial blood pressure by nictoine in rings*	*Vasopressin content in μu/ml of blood collected before and after placing nicotine* — *In rings* Before	During	Ratio	*Rostral to rings* Before	During	Ratio	*Caudal to rings* Before	During	Ratio
1	Overlapping trap. bodies	2.0	No effect	13	13	1.0				77	199	2.7
2*	" "	3.0	Rise: 8 mmHg	89	65	0.8						
3	1.5	5.5	Rise: 10 mmHg	20	24	1.2				24	385	16.0
4	2.0	6.0	No effect	38	32	0.8						
5	2.5	6.5	Fall: 38 mmHg	23	200	8.7	18	10	0.6			
6	3.0	7.0	Fall: 90 mmHg	16	107	6.7						
7	4.5	8.5	Fall: 74 mmHg	37	248	6.7	25	25	1.0			
8*	5.0	9.0	Fall: 40 mmHg	52	334	6.4						
9	5.0	9.0	Fall: 16 mmHg	20	449	22.4						
10	5.5	9.5	Fall: 12 mmHg	24	120	5.0	27	29	1.1			
11	5.5	9.5	Fall: 52 mmHg	13	128	9.8						
12	6.0	10.0	Fall: 24 mmHg	17	230	13.5	64	62	1.0			
13	9.0	13.0	Fall: 20 mmHg	35	47	1.3	62	137	2.2	62	45	0.7
14≠	5.5	9.5		95	305	3.2						
15≠	2.5	6.5		82	178	2.2						
16≠	6.0	10.0	Fall: 50 mmHg	130	300	2.3						

The arterial blood pressure changes refer to those occurring until the end of the 2 min period of blood collection after placing nicotine inside the rings. The vasopressin content refers to blood samples collected for 2 min before and 35 to 155 s after placing 80 μg (40 μg in Expts 7 and 11) nicotine in each ring, or spreading 160 μg (80 μg in Expts 7 and 11) rostral or caudal to the rings. * Expts 2 and 8 from the same cat; same expt as Figure 3. ≠ Expts 14, 15, 16, vagi cut and carotid sinuses denervated.

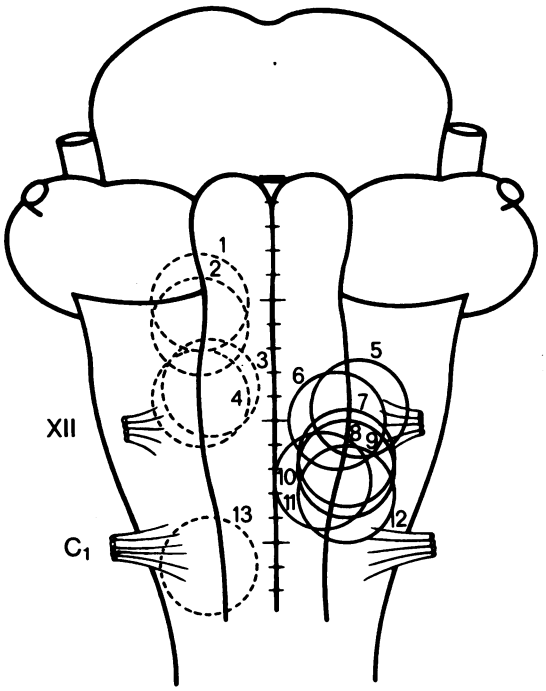

Figure 4 Diagram of ventral surface of the brain stem of the cat. The circles indicate the areas enclosed by the perspex rings in Expts 1-13 of Table 2. The number for each circle indicates the Expt No. The interrupted circles on the left refer to experiments in which no vasopressin release occurred and the full circles on the right to those in which release did occur on bilateral topical application of nicotine. C_1 rootlets of 1st cervical nerves; XII rootlets of XIIth cranial nerves. The divisions of the vertical line in the middle of the diagram correspond to 1 mm.

effective when placed in the rings, a release of vasopressin occurred when the nicotine was spread just caudal to them. Finally, in Expt 13 in which the rings were placed too far caudally for nicotine to be effective when placed in the rings, a release of vasopressin occurred when nicotine was spread rostral but not when spread caudal to them.

In none of the 13 experiments was there any detectable release of oxytocin on topical application of nicotine. For example, in Expt 9 in which the concentration of vasopressin in the blood rose to 449 μu/ml, that of oxytocin was < 14 μu/ml. This represents a vasopressin : oxytocin ratio of at least 30 : 1.

The application of nicotine to the regions from which the vasopressin release was obtained produced a fall in arterial blood pressure, whereas the application more rostrally either did not change the blood pressure, or produced a small rise. In column 4 of Table 2, the blood pressure effects are given in mmHg for 11 of the 13 experiments in which a change occurred. The values shown in this column apply only to the changes which occurred from the moment of application of nicotine in the rings to the end of the 2 min period of collection of the blood samples. The values therefore do not always give the full changes in blood pressure which took longer to develop. Although no detailed study was made of the regions from which the depressor effect was obtained its rostral border seemed to coincide with the rostral limit, but its caudal border seemed to extend beyond the caudal limit of the region from which a vasopressin release was obtained with nicotine.

The fall in arterial blood pressure cannot be the cause of the vasopressin release produced by the topical application of nicotine, although in some experiments it may have contributed to it. In Expts 5-13, the fall in blood pressure until the end of the collection of the blood samples varied between 12 and 90 mm but there was no correlation between the degree of the fall and the increase in vasopressin content of the blood. For instance, the largest release of vasopressin occurred in Expt 9 in which the fall was only 16 mm. In Expts 6, 7 and 11, the fall was 90, 74 and 52 mm respectively and could have contributed to the release. In the other experiments it was less than 40 mm and, as shown in Figure 2, a fall of this magnitude occurred after small intraventricular doses of nicotine without release of vasopressin. Moreover, in some experiments it was found that the vasopressin release did not occur with the second or third nicotine application given at hourly intervals, perhaps due to some tachyphylaxis, but the fall in blood pressure was still produced. For instance, in Expt 8, a second application of 1 mg nicotine no longer released vasopressin, but the blood pressure fell 10 mm more than during the first application which had increased the vasopressin content of the blood 6.4-fold; so it is unlikely that this vasopressin release was due to the fall in blood pressure.

In Expts 14, 15 and 16, the carotid sinuses were denervated and the vagi were cut. As this procedure often, though not always, increases the vasopressin content of the blood (Clark & Rocha e Silva, Jr, 1967) the high control values in the three experiments are readily explained. Nicotine nevertheless retained its ability to release vasopressin. With the subsequent topical application of nicotine the vasopressin in the blood rose to the same levels as in the experiments in which these nervous connections had not been interrupted. Naturally, this rise no longer meant an increase of

between 5 and 13.5-fold, but of between 2.2 and 3.2-fold. The finding that denervation of the carotid sinuses and cutting the vagi did not abolish the vasopressin release produced by topical application of nicotine is further evidence for the conclusion that the release occurs independently of the blood pressure effect.

In the experiments of Table 2, the amounts of nicotine placed in each ring were 80 or 40 μg. In three experiments the effects of smaller doses were tested as well. In Expt 6, 20 μg placed in each ring did not increase the vasopressin content of the blood, but in Expt 7, this dose caused an increase from 64 to 122 μu/ml, and 10 μg caused an increase from 55 to 95 μu/ml. Finally, in Expt 10, placing 5 μg nicotine in each ring was sufficient to increase the vasopressin content of the blood from 50 to 142 μu/ml.

Discussion

The present experiments show that the release of vasopressin without oxytocin which occurs on injection of nicotine into the cerebral ventricles of anaesthetized cats was not due to an action on the paraventricular nuclei in the ventricular walls, but that the nicotine had to enter the subarachnoid space through the lateral recesses before it could act and exert its vasopressin releasing effect. This became evident when it was found that nicotine injected into the cerebral ventricles after cannulation of the aqueduct no longer produced the vasopressin release, whereas it produced such a release when injected into the cisterna magna, from which it does not enter any part of the ventricular system. This suggested the dorsal or ventral surface of the brain stem as the site of action, the ventral surface being the more likely one as it is reached more readily by the intraventricular route. This site was confirmed when the nicotine was applied bilaterally through perspex rings to the exposed ventral surface of the medulla and again produced release of vasopressin without oxytocin. These experiments, however, do not exclude the possibility of an additional site on the dorsal surface.

The amount of nicotine required to produce vasopressin release on intraventricular injection was at least 0.5 mg and usually 1 mg injected in a volume of 0.1 ml. Some dilution would occur during the passage of the drug to the ventral surface. On topical application, 160 μg were sufficient to produce a strong release, and 10 μg was the threshold dose. These doses were applied in a volume of 40 μl, i.e. in a concentration of 1/250 and 1/4000 respectively, but it has to be realised that only a fraction of the applied nicotine will penetrate the ventral surface.

By placing the perspex rings at different sites on the ventral surface, the rostral and caudal border of the nicotine-sensitive area were found to be restricted in a longitudinal direction to between 6 and 9 mm caudal to the trapezoid bodies, that is, roughly between the origins of the rootlets of the 12th cranial and 1st cervical nerves.

There was the possibility that the nicotine injected intraventricularly might reach the supra-optic nuclei from the base of the brain after having passed through the lateral recesses into the subarachnoid space and then act directly on these nuclei which are known to release vasopressin without oxytocin on electrical stimulation. This possibility does not apply to the release on topical application because the nicotine-sensitive area is strictly circumscribed and remote from these nuclei. We must therefore assume that nicotine applied topically, and probably also when injected intraventricularly, does not act on the neurosecretory cells directly, but is stimulating them reflexly.

The afferent pathway of the milk-ejection reflex from the mammary glands to the oxytocin releasing cells of the paraventricular nuclei has been worked out in detail and defined almost in its entirety (Cowie & Tindal, 1971). Much less is known about the reflex which controls the release of vasopressin in response to changes in blood volume and distribution (for reviews see Bisset, 1975; Bisset & Jones, 1975). The afferent pathway of this reflex consists of fibres arising from baro- and stretch receptors which enter the brain stem in the sinus and vagus nerves. If the nicotine acts on this pathway the present experiments suggest a nicotine-sensitive synapse between the afferent fibres and their central projection to the neurosecretory cells which release vasopressin from the supra-optic and possibly also the paraventricular nuclei. However, since the sinus and vagus nerves enter the brain stem rostral to the region on which nicotine acts the afferent fibres would have to turn caudally for a short distance to reach the synapse or form a connection with it through one or more internuncial neurones. Such a synapse close to the ventral surface of the brain stem would perhaps be accessible to electrical stimulation and lesions. In this way the results obtained with nicotine may help to elucidate in more detail the neural control of vasopressin release.

The fall in arterial blood pressure which occurs on topical application of nicotine is not the cause of the vasopressin release. Not only was there no correlation between the two effects, but the vasopressin release was also obtained after the sinus and vagus nerves were cut. A fall in arterial

blood pressure produced by nicotine in anaesthetized cats, and attributed to an action on structures at the ventral surface of the brain stem had been described earlier by Armitage & Hall (1967). They obtained a strong depressor effect when nicotine was perfused from the fossa interpeduncularis to the cisterna magna.

As the bilateral area from which vasopressin release was obtained with nicotine lies 6-9 mm caudal to the trapezoid bodies the area is situated more caudally on the ventral surface of the medulla than the bilateral area from where most of the blood pressure effects have been obtained with drugs topically applied through perspex rings (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973; Bousquet & Guertzenstein, 1973; Edery & Guertzenstein, 1974; Guertzenstein & Silver, 1974). For pentobarbitone sodium, glycine and clonidine, it was shown that they no longer produced their depressor effects with the rings placed so that their rostral border was 5 mm caudal from the trapezoid bodies, and the glycine-sensitive area was restricted in longitudinal direction to between 1 and 2.5 mm caudal to the trapezoid bodies. Nicotine was shown in the present experiments to produce depressor effects from more caudally situated regions. Cholinomimetic substances seem to be effective also from regions extending more caudally than the pentobarbitone sodium-, glycine-, and clonidine-sensitive area. Loeschcke and his co-workers distinguished on the ventral surface lateral to the pyramid, three chemosensitive zones from which respiratory effects, and sometimes blood pressure effects as well, were obtained on electrical stimulation and on topical application of drugs or solutions with an abnormal pH. The three areas were given the letters M, S and L, standing for Mitchell, Schlaefke and Loeschcke (Trouth, Loeschcke & Berndt, 1973a). The area M lies on the trapezoid bodies. The area S lies about 2.5 to 5 mm caudal to the trapezoid bodies. It corresponds to an area in which Petrovický (1968) found groups of nerve cells immediately under the pia mater within the marginal glia, i.e. in close proximity to the liquor. Both areas are situated more rostrally than the area from which vasopressin release was obtained. The area L on the other hand, lies 5.5 to 9 mm distal to the trapezoid bodies and corresponds to the region from where vasopressin release was obtained. According to Trouth *et al.* (1973b), this area contains groups of large multipolar ganglion cells close to the ventral surface which could be the morphological substrate for its 'chemosensitivity' including that for nicotine.

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