Brit. J. Pharmacol. (1957), **12**, 461.

THE EFFECTS OF NICOTINE, HEXAMETHONIUM AND ETHANOL ON THE SECRETION OF THE ANTIDIURETIC AND OXYTOCIC HORMONES OF THE RAT

BY

G. W. BISSET* AND J. M. WALKER

From the Department of Pharmacology, University of Oxford

(RECEIVED MAY 23, 1957)

The actions of nicotine, hexamethonium, and ethanol on the hypothalamo-hypophysial system have been investigated in the rat. The antidiuretic action of nicotine was not inhibited by ethanol, nor by doses of hexamethonium which were sufficient to block both its pressor and convulsant actions. Hexamethonium itself had an antidiuretic action the mechanism of which has been investigated. Nicotine caused a release of oxytocin into the blood which was not blocked by ethanol nor significantly reduced by hexamethonium. The results suggest that any synapse which exists at the supraoptic nuclei is dissimilar in its pharmacological properties to synapses at autonomic ganglia.

Antidiuretic hormone (ADH) and oxytocin are released in response to stimulation of the central end of the vagus and to stimulation of sensory nerve endings during coitus and suckling (see Harris, 1955). From the work of Pickford and her colleagues (Pickford, 1939, 1947) it appears likely that neurohormonal reflexes of this nature involve a synapse at the supraoptic nuclei at which acetylcholine acts as the chemotransmitter of afferent nerve impulses, the supraoptico-hypophysial tract constituting the efferent pathway for the release of hormones from the neurohypophysis. That the system is analogous to synapses at autonomic ganglia or the adrenal medulla is suggested by the finding that nicotine has an antidiuretic action which is abolished by hypophysectomy (Burn, Truelove and Burn, 1945). In this investigation an attempt has been made to block the antidiuretic and chloruretic actions of nicotine in the rat by means of the ganglion-blocking agent hexamethonium. A preliminary account of this work has been given (Bisset and Walker, 1953).

The action of ethanol as a blocking agent has also been investigated since it has been shown that, although it inhibits the release of ADH in response to intravenous injection of acetylcholine (van Dyke and Ames, 1951), emotional stress (Ames and van Dyke, 1952) and intracarotid infusion of hypertonic saline (Dicker, 1954), it does not block the antidiuretic action of nicotine in man (Eggleton, 1949).

A parallel investigation has been carried out to determine the concentration of oxytocin in jugular venous blood after the injection of hexamethonium and nicotine into rats under ethanol anaesthesia.

METHODS

Experiments on Water and Chloride Excretion

The antidiuretic and chloruretic activities of drugs were compared by using Burn's method of assay of pituitary (posterior lobe) extract (PPLE) (Burn, 1937). In two series of cross-over experiments, 16 rats were given 5 ml./100 g. of either warmed tap water or 10% ethanol (v/v) by stomach tube. The rats were divided into 4 groups of 4 and each group was placed in a separate metabolism cage. Excretion of urine was measured at intervals of 15 min. until three consecutive readings differed by less than 1.5 ml. (Taylor and Walker, 1951). The time to maximal rate of excretion, as defined by Burn (1937), was calculated for each group.

The following drugs were injected 10, 25, or 30 min. after the water load or ethanol: nicotine hydrogen tartrate 0.5 or 0.75 mg./100 g. subcutaneously; hexamethonium bromide 0.5 or 5.0 mg./100 g. intraperitoneally; PPLE (Pituitrin: Parke, Davis) 6.0 mU./100 g. subcutaneously or intraperitoneally.

Control groups received corresponding injections of saline.

Urinary chlorides were estimated by Volhard's method.

Assay of Oxytocin in Blood

Male rats, 200 g. to 300 g., were anaesthetized with 5 ml./100 g. of 15% to 20% (v/v) ethanol by

*Present address: Department of Pharmacology, Charing Cross Hospital Medical School, London.

stomach tube, supplemented, if necessary, with 1 to 4 ml. of 20% ethanol by intraperitoneal injection. As soon as surgical anaesthesia had been obtained, usually within 30 min., hexamethonium or nicotine was injected and, either 5 or 10 min. later, blood was collected from the cut external jugular veins. One minute before collection, heparin *B.P.*, 4.0 units/100 g., was injected into the saphenous vein.

As a result of further experiments on a non-specific factor occurring in blood extracts (see Results), the use of polythene syringes and beakers was adopted for the withdrawal and collection of blood.

Extraction and assay of oxytocin were carried out by the method of Bisset and Walker (1954). In every instance, except where the total activity was very small, extracts were treated with sodium thioglycollate (Van Dyke, Chow, Greep, and Rothen, 1942), and it was assumed that any activity persisting after this treatment was due not to oxytocin but to some non-specific factor. Non-specific activity was present principally in those experiments in which glassware was still in use to collect blood, and in these cases the concentration of oxytocin was estimated as the difference in activity before and after treatment with thioglycollate. The validity of this procedure was established by previous work (Bisset and Walker, 1954).

In the assays, Pituitrin (Parke, Davis) was used as the standard.

RESULTS

Experiments on Water and Chloride Excretion

The Effect of Hexamethonium on the Antidiuretic and Chloruretic Actions of Nicotine.—Table I gives mean times to maximal rates of excretion. The time in control groups was 93 min. The effect of nicotine was to prolong the time to 151 min. There was no difference in effect between the two doses of 0.5 and 0.75 mg./100 g. In groups in which nicotine was preceded, at an interval of 20 min., by hexamethonium (0.5 mg./100 g.), the time was 142 min., which is not significantly less than in groups receiving nicotine alone. The conditions were similar to those in which hexamethonium abolishes the convulsant action of nicotine in the rat (Laurence and Stacey, 1952). Hexamethonium itself in a dose of 0.5 mg./100 g. had a small antidiuretic effect, prolonging the time to 109 min., which differs significantly from the control ($P = <0.001$). A tenfold increase in the dose of hexamethonium to 5.0 mg./100 g. prolonged the time to 167 min., which exceeds that observed after nicotine and is equivalent to the effect of 6.0 mU. PPLE/100 g. In groups in which the larger dose of hexamethonium was injected 5 min. before nicotine the time was increased to 204 min., which is significantly greater than after nicotine alone ($P = <0.001$) or hexamethonium alone ($P = <0.001$), suggesting not an antagonism but a summation of effects. The same dose of hexamethonium injected 5 min. before PPLE increased the time from 172 to 215 min. ($P = <0.01$ and >0.001), showing that hexa-

TABLE I

THE ANTIDIURETIC ACTION OF HEXAMETHONIUM, NICOTINE, AND PPLE IN THE RAT

Doses of drugs are expressed as quantities/100 g. of body weight. Hexamethonium (0.5 mg.) was given 10 min., hexamethonium (5.0 mg.) 25 min., and nicotine and PPLE 30 min., after the water load. PPLE = pituitary (posterior lobe) extract [Pituitrin].

Drug	Time to Maximal Rate of Excretion (min.)		
	Mean	S.E.	No. of Groups
Saline (control)	93	1·19	24
Nicotine (0·5 or 0·75 mg.)	151	2·47	20
Hexamethonium (0·5 mg.)+ nicotine	142	4·35	4
Hexamethonium (0·5 mg.)	109	2·02	4
,, (5·0 ,,)	167	4·16	16
PPLE (6·0 mU.)	172	4·21	12
Hexamethonium (5·0 mg.)+ nicotine	204	3·04	20
Hexamethonium (5·0 mg.)+ PPLE	215	13·28	4

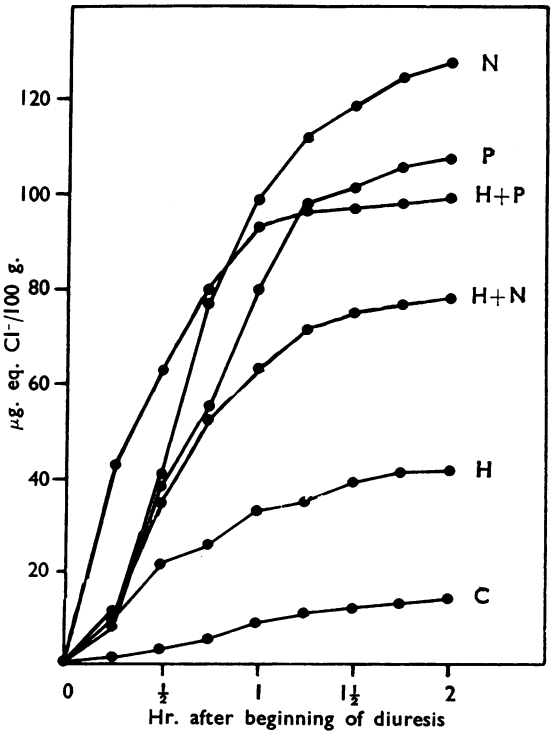

FIG. 1.—Chloruretic action of hexamethonium, nicotine, and PPLE. The chloride excretions were estimated on the groups of rats referred to in Table I. C, saline control; H, hexamethonium, 5.0 mg./100 g.; N, nicotine, 0.75 mg./100 g.; P, PPLE, 6.0 mU./100 g. PPLE = pituitary (posterior lobe) extract [Pituitrin].

methonium augments the antidiuretic action of nicotine and PPLE to a similar degree.

In the same experiments rates of urinary excretion of chloride were determined, with the results shown in Fig. 1. Both nicotine and PPLE produced a large increase in chloride excretion, but hexamethonium, although equipotent to both these drugs in its antidiuretic action, produced only a fraction of their chloruretic effect. In groups which received both hexamethonium and nicotine, the total chloruretic effect was intermediate between their effects when given separately. Hence hexamethonium increases the antidiuretic action of nicotine but reduces its chloruretic action. The fact that hexamethonium does not reduce the chloruretic action of PPLE suggests the possibility that its antagonism towards nicotine is due, not to a peripheral renal or vascular effect, but to a central inhibition of the release of a chloruretic factor or factors from the neurohypophysis.

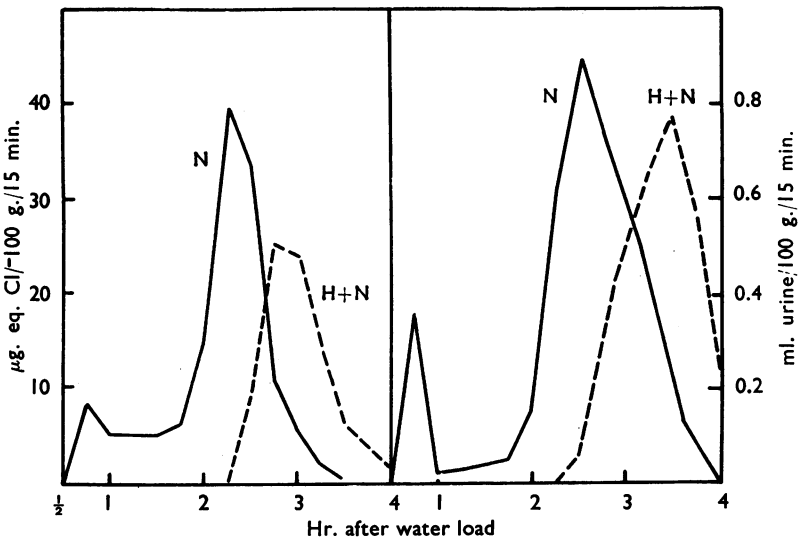

FIG. 2.—The effect of hexamethonium on the chloruretic and antidiuretic actions of nicotine. H, hexamethonium, 5.0 mg./100 g.; N, nicotine, 0.75 mg./100 g.

Fig. 2 compares the course of water and chloride excretion after nicotine alone (N) and nicotine and hexamethonium (H+N). Burn *et al.* (1945) observed that two peaks occur in a water diuresis curve after nicotine. Fig. 2 confirms this observation and shows also that two similar peaks occur in the curve of chloride excretion. The first peak, which in each case is reproducible by tyramine, and is attributed to the pressor action of nicotine, is abolished by hexamethonium; that this drug does in fact block the pressor action of nicotine has been confirmed by direct measurement of the blood pressure in a rat under similar experimental conditions. The second peak is shifted to the right, showing that the antidiuretic effect is increased. Fig. 2 also shows that the excretion of chloride is less after hexamethonium. The average total chloride excretion was 157 μg. eq. Cl^-/100 g. after nicotine alone and 86 μg. eq. Cl^-/100 g. after hexamethonium and nicotine. A correction for the amount of chloride excreted during the period of the initial pressor peak in the experiments with nicotine alone amounts to only 27 μg. eq. Cl^-/100 g., reducing the total to 130 μg. eq. Cl^-/100 g. Reduction of the chloruretic action of nicotine by hexamethonium cannot, therefore, be explained solely by abolition of a pressor effect.

The Antidiuretic Action of Hexamethonium.—Three possible mechanisms were investigated. (1) Delayed absorption of water from the intestine. (2) Effect on renal circulation due to a fall in systemic blood pressure. (3) Paralysis of the autonomic nerve supply to the bladder.

To test the first possibility, the following experiment was carried out. Sixteen rats, divided into two groups A and B of total weight 2,410 and 2,480 g. respectively, were given 5 ml./100 g. of warm tap water. Twenty-five minutes later each rat in group A was given a control injection of saline, and in group B hexamethonium 5.0 mg./100 g. intraperitoneally. Ninety-five minutes later, by which time the maximal rate of excretion and therefore of absorption of the water should have been attained, the rats were killed by decapitation and the urethras quickly ligated to prevent escape of urine. The abdomens were opened and, after ligation of the oesophagus, terminal ileum and neck of the bladder, the gastro-intestinal tract and the bladder were removed from each rat and weighed. The results are given in Table II. The mean weight of the gastro-intestinal tract in the control group A was 9.0 g. ± 0.64, compared with 10.1 g. ± 0.32 in the test group B: the difference is not significant. The total weight of the bladders in group A was 3.75 g. and in B 1.64 g. At the end of the experiment when the rats were killed,

TABLE II

THE EFFECT OF HEXAMETHONIUM ON ABSORPTION OF WATER FROM THE GUT

Group A, controls. Group B, hexamethonium 5·0 mg./100 g. 25 min. after water load.

Group	Wt. of Gastro-intestinal Tracts (g.)	m.	S.E.
A	8·7 7·7 7·7 8·9 7·5 7·9 11·4 12·2	9·0	0·64
B	9·5 9·9 10·8 10·2 9·2 9·2 10·5 11·8	10·1	0·32

group A had passed a total of 54.2 ml. urine and group B 2.5 ml. It is clear that the antidiuretic effect of hexamethonium in group B was not due to delay in the absorption of the water, nor was it associated with passive distension of the bladder.

To test the second and third possibilities, blood pressure was recorded simultaneously with urine flow in a rat under ethanol anaesthesia. As the bladder was cannulated in this preparation, any antidiuretic effect observed could not have been due to paralysis of its autonomic nerve supply. It was found that each intravenous injection of hexamethonium caused an inhibition of diuresis with a concomitant fall of blood pressure. If the fall of systemic blood pressure after hexamethonium is causally related to the antidiuretic effect, it might act either by a peripheral action on glomerular filtration in the kidney or indirectly by release of ADH from the neurohypophysis.

TABLE III

THE ANTIDIURETIC AND CHLORURETIC ACTIONS OF ETHANOL, NICOTINE, AND PPLE

Doses of drugs are expressed as quantities/100 g. of body weight. Nicotine and PPLE were given 30 min. after water or ethanol.

Drug	Time to Maximal Rate of Excretion (min.)			Total Chloride Excretion (μg. eq. Cl^-/100 g.)		
	Mean	S.E.	No. of Groups	Mean	S.E.	No. of Groups
Ethanol (10% v/v; 5·0 ml.)	95	7·0	4	49	9·6	4
Water (5·0 ml.) + nicotine (0·75 mg.) ..	146	2·9	4	200	12·6	4
Ethanol + nicotine ..	187	5·7	8	149	13·4	8
Water + PPLE (6·0 mU.)	144	2·7	6	192	14·9	6
Ethanol + PPLE ..	161	6·2	6	198	13·6	6

The Effect of Ethanol on the Antidiuretic and Chloruretic Actions of Nicotine.—A series of experiments was carried out in which the water load was replaced by 10% v/v ethanol. The doses of nicotine and PPLE were the same as in the experiments with hexamethonium.

The results are given in Table III. The mean time to maximal rate of excretion in the control groups receiving ethanol alone was 95 min. The effect of replacing the water load with ethanol was to increase the time after nicotine from 146 to 187 min. ($P = <0.001$) and to reduce the total chloride excretion from 200 to 149 μg. eq. Cl^-/100 g. ($P = <0.02$): after PPLE the time was increased from 144 to 161 min. ($P = <0.05 > 0.02$), but there was no significant change in total chloride excretion.

It is possible, therefore, that ethanol, like hexamethonium, reduces the chloruretic action of nicotine by a central inhibitory effect. It increases the antidiuretic action of nicotine, although it is not itself antidiuretic, but as it similarly enhances the antidiuretic action of PPLE the effect is, in this case, probably a peripheral one.

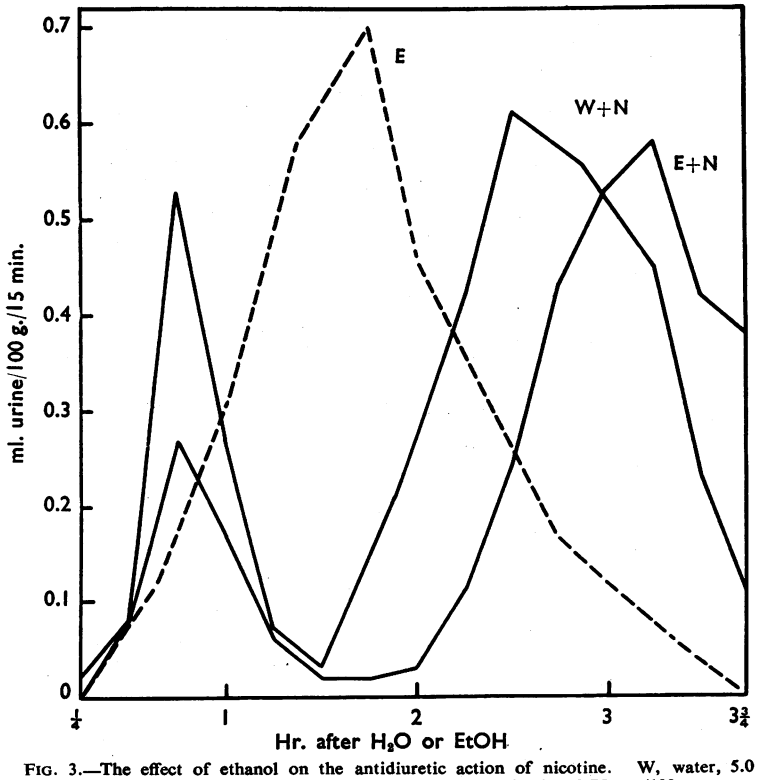

FIG. 3.—The effect of ethanol on the antidiuretic action of nicotine. W, water, 5.0 ml./100 g.; E, ethanol, 10% v/v, 5.0 ml./100 g.; N, nicotine, 0.75 mg./100 g.

Fig. 3 is included for comparison with Fig. 2. In the curves for both water and nicotine (W+N) and ethanol and nicotine (E+N) two peaks occur: the first peaks are coincident; in the ethanol and nicotine curve, however, the second peak is shifted to the right, showing that the antidiuretic action of nicotine has been potentiated by ethanol.

Assay of Oxytocin in Blood

Preliminary Experiments on a Non-specific Factor in Blood Extracts.—In a previous paper (Bisset and Walker, 1954) it was stated that in a number of experiments an oxytocic factor occurred in blood extracts which could not be identified with oxytocin, potassium or 5-hydroxytryptamine. Armstrong, Jepson, Keele and Stewart (1955) have since detected in human plasma a pain-producing substance (PPS) with oxytocic activity, which may be identical with bradykinin, and is formed when blood comes into contact with a water-wettable surface. The following experiment suggests that the non-specific factor encountered in our earlier experiments is a similar substance.

18.0 ml. blood was collected in a polythene syringe from the cut external jugular veins of four rats under ethanol anaesthesia. 9.0 ml. was introduced into a polythene beaker and 9.0 ml. into a glass beaker. The blood was allowed to stand for 10 min. The two samples were extracted by the usual method and assayed for oxytocic activity against PPLE. The activity of the first extract, prepared from the blood in the polythene beaker, and equivalent to 3.5 mU./ml., was abolished by sodium thioglycollate. The activity of the second extract amounted to 8.9 mU./ml., and this was reduced by treatment with thioglycollate to 5.1 mU./ml., the difference of 3.8 mU./ml. representing the content of oxytocin.

As a result of this experiment the use of polythene was adopted for the collection of blood samples. In none of 13 consecutive experiments following its use was non-specific activity encountered, although it has since recurred in occasional experiments.

Concentrations of Oxytocin in Jugular Venous Blood under Ethanol Anaesthesia.—The results obtained are set out in Table IV.

In control experiments under ethanol anaesthesia the average concentration of oxytocin in blood from the external jugular veins was 1.1 mU./ml. Non-specific oxytocic activity occurred in five of the six experiments in which glass was used to collect blood (Table IV, expts. 1 to 6) but was absent from the remaining five experiments in which glass was replaced by polythene.

TABLE IV

CONCENTRATIONS OF OXYTOCIN IN THE EXTERNAL JUGULAR VENOUS BLOOD OF RATS UNDER ETHANOL

The figures in parentheses give the volume of blood in ml./100 g. withdrawn in each experiment. The estimates for oxytocin are given in mU./ml. of jugular venous blood.

Expt. No.	Control	Nicotine	Hexamethonium + Nicotine	Hexamethonium
1	0·0	17·3 (3·1)	0·0 (1·3)	2·7 (1·3)
2	0·0	5·2 (1·6)	7·0	2·8 (1·6)
3	0·7	8·3 (2·7)	3·8	0·6 (0·9)
4	1·4	8·2 (2·0)	5·0 (1·6)	>0·5 (1·8)
5	2·5 (2·1)	9·5 (1·7)	4·5 (1·4)	0·4 (1·5)
6	1·9 (2·6)	8·6 (2·2)	>1·3 (0·9)	1·2 (1·7)
7	1·0 (1·7)	5·1 (2·0)	0·0 (1·1)	3·3 (1·2)
8	0·6 (1·9)	0·0 (1·6)	1·3 (1·0)	>0·6 (1·2)
9	1·6 (1·4)	5·4 (1·6)	6·1 (1·2)	
10	1·4 (1·3)	8·2 (1·9)	3·3 (2·3)	
11	1·5 (1·8)	3·1 (2·9)	4·5	
12		2·1 (1·8)	8·5	
13		0·0 (2·2)		
14		3·4 (2·4)		
15		11·8 (2·2)		
16		1·3 (1·7)		
17		4·1 (1·7)		
18		7·2 (1·8)		
19		3·3 (1·3)		
20		8·6 (2·0)		
21		0·0 (2·0)		
22		4·7 (1·1)		
23		6·2 (2·2)		
24		5·3		
m	1·1	5·7	3·8	1·5
S.E.	0·23	0·82	0·79	0·43

The mean concentration after nicotine was 5.7 mU./ml. oxytocin. This value is significantly higher than the mean in the ethanol controls ($P = <0.001$). In three experiments (Table IV, expts. 8, 13, and 21) oxytocin was not detected. In experiment 16 two assays of oxytocic activity were carried out. In the first, the total activity was 9.3 mU./ml., but this was not demonstrably reduced by treatment with thioglycollate. The second assay was carried out three days later: the activity was 1.3 mU./ml., and this was abolished by thioglycollate. It is possible that the preparation used for the second assay was relatively insensitive to the non-specific factor. The experiment emphasizes the importance of submitting all active extracts to the thioglycollate test. In experiments 18 and 19 the extracts, when tested on the rat anaesthetized with ethanol, caused an increase in total chloride excretion.

After hexamethonium alone the mean concentration of oxytocin was 1.5 mU./ml., which was not significantly different from the control series. In the experiments in which nicotine was given 5 min. after hexamethonium the mean concentration of oxytocin in the blood was 3.8 mU./ml. This value does not differ significantly from that obtained after nicotine alone. The results show that under these conditions hexamethonium does

not liberate oxytocin, nor does it inhibit the release of oxytocin by nicotine.

ADH is known to be released by haemorrhage (Ginsburg and Brown, 1956) and this might apply also to oxytocin. In Table IV the volume of blood withdrawn in each experiment is shown. In the whole series, comprising both control and test groups, there is some degree of correlation between this volume and the concentration of oxytocin in the blood ($r = +0.46$). However, only in the case of the nicotine group is the mean volume of blood withdrawn greater than in the control group and the difference is not significant ($P = > 0.05$). The differences in the mean concentrations shown for the various drugs are therefore likely to be due not to an accidental effect of haemorrhage but to the actions of those drugs on the neurohypophysis.

Discussion

The experiments reported in the first part of this paper showed that nicotine caused a profound antidiuresis and chloruresis in the rat. The antidiuresis was presumably due to release of ADH (Burn *et al.*, 1945), and the chloruresis might have been due to the release of oxytocin, since several workers have attributed the chloruretic effect of PPLE to its oxytocic fraction. The previous injection of hexamethonium or administration of ethanol did not inhibit the antidiuresis caused by nicotine, but did inhibit the chloruresis. Since neither of these drugs affected the chloruretic action of injected PPLE, the results suggested the possibility that they inhibited centrally the release by nicotine of oxytocin but not of ADH. This would mean in fact that under certain conditions the two hormones could be secreted independently by the neurohypophysis.

In order to approach the problem more directly, rats anaesthetized with ethanol were given the same injections of nicotine and hexamethonium as in the experiments on water diuresis, and samples of blood from the external jugular veins were assayed for oxytocic activity. It was found that the activity of the blood of animals which had received nicotine was significantly higher than that of the controls. Thus nicotine releases oxytocin from the neurohypophysis, and this release is not blocked by ethanol. However, the mean levels in the blood after nicotine alone were not significantly different from those after hexamethonium and nicotine, so that the hypothesis that hexamethonium inhibits the release of oxytocin by nicotine is not confirmed. The results suggest that the chloruretic action of nicotine is due not only to release of oxytocin but also to some other effect on renal function which is inhibited by hexamethonium.

In some experiments, assays of both hormones in the blood were carried out in an attempt to see if the ratio in which they were released was constant or variable. It appeared at first that the ratio of oxytocin to ADH was significantly higher after nicotine than in the controls, and these results have already been quoted by one of us (Walker, 1957). However, while the present paper was still in preparation, Ginsburg (1956) reported that Pituitrin, which was used in our experiments as the standard, had less antidiuretic activity in relation to its pressor and oxytocic potencies than did the international standard of PPLE, and that the activity varied greatly according to the particular rat used for the assay. The results of assays of ADH are therefore of doubtful value and they have been omitted from the tables. It is intended to repeat this part of the work using a more satisfactory standard.

It has been shown that hexamethonium does not block the release of oxytocin nor inhibit the antidiuretic action of nicotine in doses which are sufficient to block both the pressor and convulsant actions of this drug. However, the anticonvulsant action of hexamethonium is not to be taken as evidence of a central inhibitory effect, for Laurence and Stacey (1953) showed that it depends on blockage of the release of adrenaline. It is indeed possible that the drug fails to penetrate the blood-brain barrier. If, however, it does reach the anterior hypothalamus it must be concluded that any synapse which exists at the supraoptic nucleus is dissimilar in its pharmacological properties to synapses at autonomic ganglia. This is in agreement with the observations of Supek and Eisen (1953), who similarly failed to block the antidiuretic and chloruretic actions of nicotine in rats with a number of ganglion-blocking agents.

The ability of ethanol to produce a state of "functional neurohypophysectomy" (Dicker, 1954) evidently depends on the nature of the stimulus to the neurohypophysis. In this investigation, ethanol did not inhibit the antidiuretic action of nicotine, nor block the release of oxytocin. This is consistent with the finding of Ginsburg and Brown (1956) that the antidiuretic activity of blood during haemorrhage in rats under ethanol anaesthesia is only slightly lower than that under ether, pentobarbitone, or urethane.

This work was carried out during the tenure by one of us (G. W. B.) of a Scholarship for Training in Research Methods, for which he wishes to express his gratitude to the Medical Research Council.

References

Ames, R. G., and van Dyke H. B. (1952). *Endocrinology*, **50**, 351.

Armstrong D., Jepson J. B., Keele, C. A., and Stewart, J. M. (1955). *Nature, Lond.*, **174**, 791.

Bisset, G. W., and Walker. J. M. (1953). *Abstr. XIX int. physiol. Congr., Montreal*, 1953, p. 251.

—— —— (1954). *J. Physiol.*, **128**, 588.

Burn, J. H. (1937). *Biologica Standardization*, 1st ed., p. 69. London: Oxford University Press.

—— Truelove, L. H., and Burn, I. (1945). *Brit. med. J.*, **1**, 403.

Dicker, S. E. (1954). *J. Physiol.*, **124**, 464.

Eggleton, M. G. (1949). Ibid., **108**, 482.

Ginsburg, M. (1956). *Brit. J. Pharmacol.*, **11**, 245.

—— and Brown, L. M. (1956). Ibid., **11**, 236.

Harris, G. W. (1955). *Neural Control of the Pituitary Gland.* London: Edward Arnold.

Laurence, D. R., and Stacey, R. S. (1952). *Brit. J. Pharmacol.*, **7**, 80.

—— —— (1953). Ibid., **8**, 62.

Pickford, M. (1939). *J. Physiol.*, **95**, 226.

—— (1947). Ibid., **106**, 264.

Supek, Z., and Eisen, V. (1953). *Arch. int. Pharmacodyn.*, **93**, 75.

Taylor, N. B. G., and Walker, J. M. (1951). *J. Physiol.*, **113**, 412.

van Dyke, H. B., and Ames, R. G. (1951). *Acta endocrinol.*, **7**, 110.

—— Chow, B. F., Greep, R. O., and Rothen, A. (1942). *J. Pharmacol.*, **74**, 190.

Walker, J. M. (1957). *The Neurohypophysis*, p. 221. London: Butterworths.