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# THE EFFECTS OF DRUGS ON THE RELEASE OF VASOPRESSIN

BY

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A wide variety of drugs have been shown to inhibit the release of neurohypophysial hormones in several species of experimental animals in response to a large number of different experimental stimuli (Grosvenor & Turner, 1957; Chaudhury, 1961; Mills & Wang, 1964b; Boris & Stevenson, 1967).

There is, however, little evidence as to the mode of action of these drugs or their site of action, although it is likely that many of them exert their inhibitory effect in the central nervous system (CNS). The purpose of the experiments described here was to investigate the effects of a number of drugs on the release of vasopressin by the three stimuli found most effective in a previous investigation (Dyball, 1966, 1968). Drugs were chosen which antagonize the action of acetylcholine, adrenaline and noradrenaline, 5-hydroxytryptamine and histamine because there is good circumstantial evidence that these substances may be chemical transmitters in the central nervous system (Crossland, 1960). Adrenaline, noradrenaline and ethanol were also investigated because of the reports that these compounds affect the release of neurohypophysial hormone (O'Connor & Verney, 1945; Verney, 1947; Duke & Pickford, 1951; Ames & van Dyke, 1952; Dicker, 1958; Chaudhury, 1961; Fuchs, 1966).

It is likely that there is more than one central nervous pathway for the release of neurohypophysial hormones (Mills & Wang, 1964a; Tindal, Knaggs & Turvey, 1967a, b) so any drug which inhibits the effects of all the stimuli used to release vasopressin is likely to act on or near the final common pathway for release. A drug which inhibits the effects of some but not all stimuli may be assumed to depress transmission along one pathway but a stimulus involving an alternative pathway would not be blocked and would still be effective. This means that a consideration of the types of drugs which inhibit neurohypophysial hormone release may provide evidence about the nature of transmission in the final common pathway involved in neurohypophysial hormone release.

## METHODS

Male albino rats approximately 150 g in weight were used throughout. Fifteen minutes after the subcutaneous injection of atropine sulphate (100 mg/kg) to prevent excessive secretion of mucus in the respiratory tract, the animals were anaesthetized with ether and cannulae filled with heparinized

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0.85% sodium chloride solution were inserted into the right femoral vein (for the injection of heparin), into the right external jugular vein (for the collection of blood from the head) and into the right common carotid artery (for the injection of the drugs). All the drugs were dissolved in 0.85% sodium chloride solution for injection.

After completion of the surgical procedures, the drugs (with the exception of reserpine which was given intraperitoneally on the previous day) were injected into the right common carotid artery and a blood sample (1 ml.) was immediately taken from the external jugular vein. The stimulus was then applied and another 1 ml. sample of blood taken from the external jugular vein approximately 30 sec after the end of the stimulus.

Three stimuli which had been found to be consistently effective in releasing vasopressin in the experimental conditions used (Dyball, 1966, 1968) were employed. (1) Electrical stimulation of the central end of the severed right vagus nerve (stimulation for 30 sec with a break of 2 sec between the fifteenth and seventeenth sec at 15 V with square waves of 2 msec duration and 100 c/s); (2) injection of 0.1 M calcium chloride solution to 6.3 ml./kg (at a rate of 0.6 ml./min) into the right common carotid artery (=approximately 25 mg $Ca^{++}$/kg); (3) haemorrhage. This consisted of taking 6 ml. of blood in three 2 ml. samples from the right external jugular vein and took approximately 4 min. The first 2 ml. sample was treated as the "before stimulation" sample and the third 2 ml. as the "after stimulation" sample.

The samples of whole blood were assayed for antidiuretic activity immediately after collection by a method derived from that of Jeffers, Livezey & Austin (1942) using the apparatus described by Dyball, Lane & Morris (1966) to maintain the water load of the assay animal and to record the rate of urine flow.

The results of the antidiuretic assays are expressed in $\mu$-u./ml. arginine vasopressin (Tonephin, Hoechst Ltd.).

The following drugs and hormones were used: adrenaline hydrochloride (Parke Davis Ltd.); chlorpheniramine maleate* (Piriton, Allen and Hanburys Ltd.); chlorpromazine hydrochloride* (Largactil, May and Baker Ltd.); chlorpromazine *N*-oxide acid maleate (May and Baker Ltd.); ethopropazine hydrochloride (May and Baker Ltd.); mecamylamine hydrochloride* (Merck, Sharp and Dohme Ltd.); *l*-noradrenaline bitartrate* (Levophed, Bayer Ltd.); pempidine tartrate (May and Baker Ltd.); phenoxybenzamine hydrochloride (Dibenyline, Smith, Kline and French Ltd.); promethazine hydrochloride (Phenergan, May and Baker Ltd.); reserpine* (Serpasil, Ciba Ltd.); trimeprazine tartrate (May and Baker Ltd.); tyramine hydrochloride (Koch-Light Ltd.).

Drugs marked with an asterisk were obtained as injectable solution; the other drugs were dissolved in 0.85% sodium chloride for injection.

In addition, a series of synthetic acetylcholine antagonists (described in this paper as compounds A to F) which were supplied by Professor P. Pratesi of the University of Pavia, Italy, were investigated. Their chemical names are as follows: Compound "A," *N*-$\beta$-(dicychlohexlaminoethyl) piperidine dihydrochloride; Compound "B," *N*-methyl-*N*-($\beta$-dicyclohexalaminoethyl piperidinium bromide; Compound "C," *N*-methyl-*N*-(3:3 diphenylpropyl)piperidinium iodide; Compound "D," *N*-($\beta$-diphenylaminoethyl)triethylammonium bromide; Compound "E," *N*-($\beta$-diphenylaminoethyl)-piperidine phosphoric acid; Compound "F," *N*-methyl-*N*-($\beta$-diphenylaminoethyl)piperidinium bromide.

Compound B has considerable atropine-like activity: for example, it inhibits the contraction of isolated strips of rat intestine after application of acetylcholine and prevents chromodacryorrhea after intraperitoneal injection of carbachol (Grumelli & Cattaneo, 1961). The other compounds have similar pharmacological properties (Pratesi, 1964, 1965, personal communications).

## RESULTS

Table 1, which summarizes some results reported in a previous paper (Dyball, 1968), shows that the atropine sulphate used for premedication of the experimental animals before the induction of the ether anaesthesia did not affect the release of vasopressin

TABLE 1

EFFECTS OF HAEMORRHAGE, CALCIUM CHLORIDE INJECTION AND VAGAL STIMULATION ON THE BLOOD CONCENTRATION OF ANTIDIURETIC HORMONE IN THE ABSENCE OF DRUGS

Results expressed in terms of μ-u./ml. arginine vasopressin.

| Haemorrhage | | Calcium chloride injection | | Vagal stimulation | |
|---|---|---|---|---|---|
| Before | After | Before | After | Before | After |
| <125 | 593 | 50 | 580 | <200 | 2,475 |
| <125 | 373 | <100 | 400 | <50 | 665 |
| <125 | 500 | 100 | 215 | <50 | 238 |
| <125 | 880 | 32 | 537 | <50 | 623 |
| <125 | 555 | <100 | 250 | <200 | 1,366 |
| <125 | 3,337 | <100 | 444 | 364 | 1,888 |
| <125 | 793 | 63 | 4,029 | <200 | 434 |
| ( — | 510) | 309 | 5,380 | <100 | 384 |
| | | 249 | 325 | | |
| | | (388 | 720) | | |
| | | ( — | 696) | | |

by the three stimuli chosen. All the results shown in the tables are expressed as μ-u./ml. of arginine vasopressin in the blood samples.

Table 2 shows the effects of the drugs investigated on the release of vasopressin: Table 3 is a summary of Table 2.

In each experiment a blood concentration of vasopressin of 250 μ-u./ml. and above was considered as an experiment in which release of antidiuretic hormone (ADH) had occurred and a value below this as one in which release had been inhibited. On this basis, each group of experiments was given a score of a number of experiments in which release had occurred compared with the total number of experiments. These are the figures shown in Table 3. In a few experiments, for reasons which are not clear, the initial blood concentration of ADH was higher than 250 μ-u./ml. In these instances, if the concentration after stimulation was at least double that before, a release was still considered to have occurred. If the concentration after stimulation was not at least double that before stimulation it was considered possible that the release might not have been inhibited, but that the initial concentration was so high that further stimulation could not raise the blood concentration further, and the result was discarded. The high initial values for blood ADH concentration occasionally encountered may have been caused by the effects of the ether anaesthesia, the stress of the surgical procedures, a drop in blood pressure as a result of the injection of some of the drugs or by a combination of all these factors.

Table 3 shows that all the acetylcholine antagonists except hexamethonium, which probably does not cross the blood–brain barrier, had some effect on the release of vasopressin by calcium chloride. In addition all, with the exception of hexamethonium and pempidine, inhibited release by vagal stimulation but not by haemorrhage.

Adrenaline inhibited release of vasopressin by calcium chloride in three out of four experiments and by vagal stimulation in three out of seven experiments. Noradrenaline and tyramine had no consistent effect on the release of vasopressin but phenoxybenzamine inhibited release after calcium chloride injection in five out of the eight experiments.

TABLE 2

EFFECTS OF DRUGS ON THE BLOOD CONCENTRATION OF VASOPRESSIN AFTER STIMULATION WITH HAEMORRHAGE, CALCIUM CHLORIDE INJECTION AND VAGAL STIMULATION

Results expressed in terms of μ-u./ml. arginine vasopressin. The figures in brackets represent experiments considered invalid by criteria described in the text.

| | Haemorrhage | | Calcium chloride injection | | Vagal stimulation | |
|---|---|---|---|---|---|---|
| Drug | Before | After | Before | After | Before | After |
| Pempidine tartrate (13·3 mg/kg) | 91 | 396 | <100 | <100 | 103 | 449 |
| | 100 | 2,100 | <100 | 721 | 38 | 400 |
| | 200 | 1,238 | <100 | <100 | 314 | 2,371 |
| | 134 | 4,250 | <100 | 135 | <200 | 1,526 |
| | | | | | 100 | 78 |
| | | | | | <100 | 353 |
| Mecamylamine hydrochloride (10 mg/kg) *(6·6 mg/kg) | 182 | 911* | <50 | <50 | (635 | 483) |
| | 661 | 3,200* | <100 | 406 | 19 | 184 |
| | 59 | 504 | 44 | 49 | 100 | 148 |
| | 195 | 1,838 | <100 | <100 | 100 | 77* |
| | 83 | 3,584 | 219 | 353 | (277 | 534) |
| | | | (593 | 606) | | |
| | | | 105 | 200 | | |
| | | | 220 | 179 | | |
| | | | 189 | 101 | | |
| Hexamethonium bromide (16·6 mg/kg) | <100 | 93 | 226 | 428 | ( — | 419) |
| | 800 | 7,351 | 334 | 830 | 187 | 8,232 |
| | (538 | 553) | (314 | 400) | 177 | 263 |
| | 18 | 889 | 283 | 620 | 178 | 430 |
| | 98 | 1,467 | 102 | 324 | 13 | 105 |
| | 317 | 759 | 32 | 77 | 166 | 9,704 |
| | | | 476 | 2,370 | | |
| | | | ( — | 3,044) | | |
| Compound A (33·3 mg/kg) | <100 | 532 | 87 | 137 | (325 | 237) |
| | <100 | 613 | 81 | 400 | <200 | 185 |
| | 50 | 499 | <200 | 47 | <200 | 80 |
| | <100 | 8,000 | <50 | 73 | <200 | 608 |
| | | | | | <50 | 50 |
| Compound B (13·3 mg/kg) | <100 | 385 | <100 | 79 | <100 | <100 |
| | <100 | 1,038 | 68 | 109 | 33 | 51 |
| | <100 | 1,473 | <25 | <25 | <100 | <100 |
| | 77 | 1,465 | <100 | <100 | (400 | 100) |
| | | | 84 | 30 | <100 | <100 |
| | | | 119 | 1,131 | | |
| Compound C (6·6 mg/kg) *(13·3 mg/kg) | <100 | 886 | <100 | 213 | <200 | <100 |
| | <200 | 702 | <100 | 200 | 126 | 352 |
| | <100 | 4,616* | <100 | 47 | <100 | 111 |
| | 135 | 596 | 159 | 100 | 216 | 912 |
| | <100 | 598 | | | 10 | 216 |
| | 287 | 1,556 | | | 96 | 271 |
| | | | | | 67 | 227 |
| | | | | | 13 | 6 |
| | | | | | <100 | 120 |
| Compound D (5 mg/kg) | (877 | 1,063) | < 50 | 91 | 78 | 117 |
| | 74 | 1,022 | <50 | 53 | <100 | 67 |
| | <100 | 215 | <50 | 50 | 55 | 78 |
| | ( — | 2,934) | 179 | 254 | 13 | 1 |
| | 250 | 2,202 | 24 | 58 | <50 | 55 |
| | <100 | 38 | <100 | 184 | | |
| | 270 | 1,425 | | | | |
| Compound E (10 mg/kg) *(20 mg/kg) **(6·6 mg/kg) | 139 | 1,182* | <100 | 256 | <100 | 65** |
| | ( — | 817*) | <100 | 27 | <100 | 860** |
| | 93 | 1,052 | <100 | <100 | <100 | 353** |
| | <100 | 2,204 | <100 | 105 | 52 | 140 |
| | | | | | <100 | 235 |

TABLE 2—*Continued.*

| Drug | Haemorrhage | | Calcium chloride injection | | Vagal stimulation | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| Compound F (5 mg/kg) | 96 | 1,680 | 113 | 103 | 121 | 147 |
| | 63 | 1,545 | 95 | 119 | <100 | <100 |
| | <100 | <100 | 66 | 79 | <50 | <50 |
| | <200 | 1,519 | 86 | 375 | 53 | 50 |
| | 97 | 1,899 | 61 | 98 | <50 | 134 |
| | 377 | 4,271 | <100 | <100 | <50 | 134 |
| Adrenaline hydrochloride (0·0066 mg/kg) | 67 | 1,434 | <100 | 149 | 75 | 63 |
| | 111 | 5,528 | <100 | 162 | <100 | 167 |
| | 100 | 498 | <100 | 254 | 86 | 1,293 |
| | 164 | 2,154 | 32 | 144 | 224 | 1,340 |
| | | | | | 263 | 738 |
| | | | | | <100 | 228 |
| | | | | | 280 | 2,505 |
| Tyramine hydrochloride (0·133 mg/kg) | <100 | 1,883 | 24 | 264 | <100 | 1,270 |
| | 81 | 8,656 | 77 | 424 | <100 | 800 |
| | <100 | 4,450 | 124 | 981 | <200 | 1,072 |
| | <100 | 2,154 | <100 | 305 | 176 | 532 |
| | | | | | 65 | 50 |
| | | | | | <100 | 942 |
| | | | | | <100 | 1,808 |
| | | | | | <100 | 200 |
| | | | | | <100 | 509 |
| Phenoxybenzamine (1·33 mg/kg) | <100 | 724 | <100 | 112 | 74 | 2,038 |
| | 154 | 611 | 49 | 127 | 66 | 892 |
| | 173 | 2,142 | 13 | 153 | (280 | 549) |
| | (295 | 110) | 89 | 771 | <200 | 159 |
| | 169 | 451 | <100 | 74 | <200 | 2,352 |
| | (264 | 281) | 42 | 499 | <200 | 2,758 |
| | | | <100 | <100 | | |
| | | | 112 | 743 | | |
| Noradrenaline bitartrate (0·0066 mg/kg) | <100 | 4,328 | <100 | 220 | <100 | 834 |
| | <100 | 2,237 | 100 | >1,600 | <100 | 785 |
| | <100 | 2,946 | <100 | 240 | <200 | 1,685 |
| | 50 | 2,450 | <100 | 831 | <50 | 50 |
| | <100 | 220 | 11 | 733 | <100 | 317 |
| | | | <100 | 261 | <100 | 1,151 |
| | | | 200 | 842 | <100 | 1,017 |
| | | | | | <100 | 2,537 |
| Chlorpromazine hydrochloride (5 mg/kg) *(16 mg/kg) | 129 | 3,654 | 50 | 65 | <100 | 96 |
| | 120 | 904 | ( — | 95) | <100 | 61 |
| | 158 | 2,345 | 185 | 364 | 74 | 126 |
| | 95 | 650 | 76 | 125 | ( — | 65) |
| | ( — | 1,903) | 186 | 152 | <100 | 100 |
| | 64 | 50 | 90 | 200 | 57 | 154 |
| | 11 | 22* | | | 59 | 428 |
| | 166 | 100* | | | | |
| | 58 | 117* | | | | |
| | (313 | 23)* | | | | |
| Reserpine (1 mg/kg) | <100 | 320 | <50 | 874 | <100 | 100 |
| | <100 | 907 | 90 | 285 | 50 | 50 |
| | <100 | 2,701 | <100 | 301 | <100 | 344 |
| | <100 | 1,495 | 48 | 895 | <100 | 1,276 |
| | | | | | <100 | 392 |
| Ethanol (5% body weight *per os* of a 12% aqueous solution) | <100 | 983 | <100 | 3,199 | <100 | 100 |
| | <100 | 1,276 | <25 | 58 | <100 | 100 |
| | <50 | 454 | <50 | 17 | 26 | 670 |
| | <50 | 400 | <100 | 42 | <100 | 800 |
| | <100 | 1,315 | <50 | <50 | <100 | 325 |
| | <100 | 1,234 | <50 | <50 | <100 | 149 |
| Chlorpheniramine maleate (16·6 mg/kg) | 140 | 958 | <100 | 456 | 78 | 250 |
| | ( — | 2,145) | <100 | 526 | 93 | 184 |
| | 84 | 6,723 | 41 | 222 | <100 | 620 |
| | 83 | 793 | <100 | 709 | 76 | 568 |

TABLE 3

SUMMARY OF TABLE 2 SHOWING THE EFFECTS OF DRUGS ON VASOPRESSIN RELEASE BY HAEMORRHAGE, CALCIUM CHLORIDE INJECTION AND VAGAL STIMULATION

The figures before the oblique lines indicate the number of experiments in which release was judged to have occurred (by criteria described in the text) and those after the oblique lines the total number of experiments.

| Drug | Haemorrhage | Calcium chloride injection | Vagal stimulation |
|---|---|---|---|
| Controls (no drug) | 7/7 | 8/9 | 6/7 |
| Pempidine | 4/4 | 1/4 | 5/6 |
| Mecamylamine | 5/5 | 2/8 | 0/3 |
| Hexamethonium | 4/5 | 5/6 | 4/5 |
| Compound A | 4/4 | 1/4 | 1/4 |
| Compound B | 4/4 | 1/6 | 0/4 |
| Compound C | 6/6 | 0/4 | 3/9 |
| Compound D | 3/5 | 1/6 | 0/5 |
| Compound E | 3/3 | 1/4 | 2/5 |
| Compound F | 5/6 | 1/6 | 0/6 |
| Adrenaline | 4/4 | 1/4 | 4/7 |
| Tyramine | 4/4 | 4/4 | 7/9 |
| Phenoxybenzamine | 4/4 | 3/8 | 4/5 |
| Noradrenaline | 4/5 | 5/7 | 7/8 |
| Chlorpromazine | 4/5 (at 5 mg/kg)<br>0/3 (at 16·6 mg/kg) | 1/5 | 1/6 |
| Reserpine | 4/4 | 4/4 | 3/5 |
| Ethanol | 6/6 | 1/6 | 3/6 |
| Chlorpheniramine | 3/3 | 3/4 | 3/4 |

TABLE 4

EFFECTS OF PROMETHAZINE HYDROCHLORIDE, ETHOPROPAZINE HYDROCHLORIDE, TRIMEPRAZINE TARTRATE AND CHLORPROMAZINE *N*-OXIDE ACID MALEATE ON THE CONCENTRATION OF VASOPRESSIN IN JUGULAR BLOOD AND THEIR EFFECTS ON THE SUBSEQUENT RELEASE OF VASOPRESSIN BY HAEMORRHAGE

Results expressed in terms of μ-u./ml. arginine vasopressin: each drug was given in a dose of 16·6 mg/kg

| Drug | Before injection | After injection | After subsequent haemorrhage |
|---|---|---|---|
| Promethazine | — | 2,053 | 3,027 |
| | — | 422 | 2,262 |
| | <400 | 1,400 | 1,313 |
| | 91 | 761 | 3,091 |
| | 507 | 1,355 | 962 |
| | 26 | 385 | 998 |
| | <100 | 400 | 460 |
| Ethopropazine | <100 | 177 | 120 |
| | <100 | 178 | 630 |
| | 67 | 130 | 57 |
| | <100 | 196 | 400 |
| | 60 | 500 | 400 |
| | 44 | 73 | — |
| Trimeprazine | <100 | 438 | 168 |
| | <100 | <100 | <100 |
| | 61 | 74 | 70 |
| | 34 | 48 | 39 |
| | 116 | 264 | 76 |
| | <100 | 180 | 267 |
| Chlorpromazine *N*-oxide | <100 | 58 | 123 |
| | 47 | 67 | 119 |
| | <100 | <100 | <100 |
| | 67 | 106 | 65 |

Reserpine in a dose (1 mg/kg) which was sufficient to cause the animals to become very sleepy had no consistent effect on vasopressin release, nor had chlorpheniramine. Ethanol caused some reduction in vasopressin release after the injection of calcium chloride or vagal stimulation but seemed to have no effect on ADH release by haemorrhage.

Chlorpromazine (in sufficiently high doses) inhibited vasopressin release even after the stimulus of severe haemorrhage. On the other hand promethazine (see Table 4) released vasopressin when it was injected and did not prevent a further release of vasopressin by subsequent haemorrhage. The effects of three other phenothiazine derivatives were investigated in an attempt to find out which of the differences in the molecular structure of chlorpromazine and promethazine were responsible for their different actions. Chlorpromazine *N*-oxide acted like chlorpromazine. Ethopropazine acted in a similar way to promethazine but was not such an effective stimulus, and trimeprazine, although it caused some release of vasopressin, inhibited the release of vasopressin by haemorrhage.

If it is assumed that promethazine, chlorpromazine and the other phenothiazine derivatives investigated act at the same site in the central nervous system, the excitatory effects of promethazine should be consistently inhibited by chlorpromazine. Table 5 shows that in four out of four experiments, chlorpromazine did prevent the excitatory effects of promethazine.

TABLE 5

EFFECT OF CHLORPROMAZINE ON THE RELEASE OF VASOPRESSIN BY THE INTRA-CAROTID INJECTION OF PROMETHAZINE

Results expressed in terms of $\mu$-u./ml. of arginine vasopressin: both drugs were given in a dose of 16·6 mg/kg.

| Before promethazine | After promethazine |
|---|---|
| 67 | <100 |
| 80 | <100 |
| <100 | <100 |
| <100 | 29 |

## DISCUSSION

The work of Pickford *et al.* (Pickford, 1939, 1947; Duke & Pickford, 1951; Duke, Pickford & Watt, 1950) strongly suggests that the nervous mechanism for the release of neurohypophysial hormones is cholinergic. Pickford and her co-workers, Fang, Lin & Wang (1962) and Mills & Wang (1964b) found, however, that atropine did not prevent the release of vasopressin. Table 1 shows that atropine did not prevent vasopressin release by the three stimuli used in this investigation. Tables 2 and 3 show that all the other acetylcholine antagonists investigated had some effect on the release of vasopressin with the exception of hexamethonium (which probably does not cross the blood–brain barrier). Pempidine inhibited the release only by the stimulus of calcium chloride injection but mecamylamine and all the compounds prepared by Pratesi which have been shown to have atropine-like activity also inhibited release by vagal stimulation. None of these compounds prevented release of vasopressin by haemorrhage but the possibility cannot be excluded that they would have done so in higher doses, or if the haemorrhage had not been as severe. An alternative possibility is that the release of vasopressin by

haemorrhage is mediated by a nervous pathway not involving the cholinergic step which was blocked in the experiments with calcium chloride and vagal stimulation (and which presumably corresponds to the cholinergic synapse which was stimulated in the experiments of Pickford and her co-workers).

It is likely that all the acetylcholine antagonists used (except hexamethonium) cross the blood–brain barrier. Harrington, Kincaid-Smith & Milne (1958) have shown that both pempidine and mecamylamine can be extracted from brain tissue after intraperitoneal injection in the rat. It is also likely that the effects of calcium chloride and central vagal stimulation are exerted in the central nervous system so that inhibition of their effects by Pratesi's compounds is probably a central effect. This implies that they too can cross the blood–brain barrier. It is perhaps surprising that the compounds prepared by Pratesi inhibited the release of vasopressin because they have principally atropine-like properties (Grumelli & Cattaneo, 1961 ; and Pratesi, 1964, 1965, personal communications) and atropine itself does not prevent the release of neurohypophysial hormones. This finding may be comparable with that of Fang *et al.* (1962) who failed to demonstrate an inhibition of vasopressin release by atropine but could do so after administration of *N*-ethylnortropin-benzhydrylether hydrobromide.

Inspection of Table 2 suggests that pempidine and compounds A to F tended to inhibit the release of vasopressin in response to vagal stimulation and calcium chloride injection. An objective test was necessary, however, to see whether the values after stimulation in the presence of drugs shown in Table 2 differed from the control experiments. Student's *t* test could not be used to demonstrate a difference between the two groups of figures because of the small number of results with each drug and because of doubts as to whether their distribution was normal. Accordingly it was decided to use a form of simple non-parametric statistics. An arbitrary level (of 250 $\mu$-u./ml.) was set near the upper limit of the values for the blood ADH concentration before stimulation ; any value after stimulation which was above this figure was considered to be an experiment in which release had occurred and any below this as one in which release had been inhibited. Table 3 was drawn up on this basis.

This method of interpretation is not beyond criticism but, because of the very variable concentration before and after stimulation and the comparatively imprecise nature of the assays on which the table is based, a procedure of this type seems to be the only one which is justifiable. A better method of interpretation would have been to express the value after stimulation as a percentage of that before stimulation but this was not possible in experiments in which the initial level was so low that it could not be measured. Confidence in this method of interpretation was increased when it was seen that the overall picture of the results differed little when the arbitrary value was moved up or down over a wide range of values.

The high values for blood ADH concentration sometimes encountered before stimulation are likely to have been the result of the stress of the surgery, a fall in blood pressure which would have resulted from the injection of some of the drugs, ether anaesthesia or a combination of all these factors. Ether was used in spite of its action in releasing ADH because it is unlikely itself to inhibit vasopressin release and any inhibition of release is likely to have been caused by the drugs injected. The varied concentrations both before and after stimulation may be explicable in terms of

the short half-life of vasopressin in the blood and the unavoidable variation in the time interval between the end of the stimulation and the collection of the second blood sample (between 30 and 60 sec).

Some of the variation can undoubtedly be explained in terms of the inaccuracy of the assays but the point may be made that the assay method used in these experiments was no less precise than similar methods used by other workers. A typical index of precision ($\lambda$) for an antidiuretic assay was 0.133 which is comparable with $\lambda = 0.143$ obtained by Hunter, Kalant & Ogilvie (1959) and $\lambda = 0.132$ obtained by Crawford & Pinkham (1954).

The results of Weil-Malherbe, Axelrod & Tomchick (1959) suggest that adrenaline penetrates the blood–brain barrier to some extent in the hypothalamic region but not elsewhere in the central nervous system. The variable effects of adrenaline may, however, have resulted from the different extent to which it crossed the blood–brain barrier in the different experiments, and the lack of effect of noradrenaline and tyramine from their failure to cross it at all. If these compounds do cross the blood–brain barrier, however, it is not likely that either adrenaline or noradrenaline are directly involved in the release of neurohypophysial hormones.

Phenoxybenzamine almost certainly crosses the blood–brain barrier because high doses given to human subjects may cause nausea, vomiting, hyperventilation, motor excitability and convulsions (Goodman & Gilman, 1965). Its failure to prevent completely vasopressin release may mean either that there is no essential adrenergic step in the pathway for ADH release which can be blocked by phenoxybenzamine or that the dose used was too low. The lack of effect of reserpine which is known to reduce the concentrations of noradrenaline and 5-hydroxytryptamine in the central nervous system may similarly have been caused either by insufficient dosage or by the absence of a vital step in the pathway for vasopressin release which depends on 5-hydroxytryptamine or noradrenaline as a transmitter substance.

Although ethanol tended to prevent vasopressin release, it is not likely to depress an essential pathway for the release specifically because its effects are inconsistent. Its action is more likely to be due to a general depression of hypothalamic activity although its effects may be the result of interference with cholinergic transmission as suggested by Dicker (1958).

Finally it was decided to test the effects of antihistamine substances. Chlorpheniramine (which can cause drowsiness and sometimes signs of central nervous stimulation if given to human subjects in high doses, which suggests that it probably does cross the blood–brain barrier) was ineffective in preventing the release of vasopressin. This suggests that the effect of promethazine of releasing vasopressin is not the result of its antihistamine properties.

Chlorpromazine in sufficiently high doses prevented the release of vasopressin by all the three stimuli. An attempt was therefore made by investigating the effects of three other phenothiazine derivatives to establish which features of the molecules of promethazine and chlorpromazine were responsible for their dissimilar actions (see Table 4).

Ethopropazine caused some release of vasopressin in five out of six experiments and inhibition of release by subsequent haemorrhage in two out of five experiments. Trimeprazine caused some release of vasopressin in three out of six experiments and inhibited

release by haemorrhage in five out of six experiments. Chlorpromazine *N*-oxide did not itself cause a release of vasopressin but prevented release by haemorrhage in four out of four experiments.

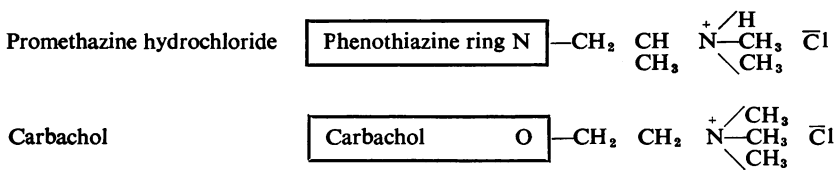
The effects of chlorpromazine on the release of vasopressin by promethazine was also investigated. Prevention by chlorpromazine of the release of vasopressin by promethazine would make it likely that both drugs were exerting their effects on the same mechanism and that these effects were comparatively specific. Table 5 shows that chlorpromazine prevented the release of vasopressin by promethazine in four out of four experiments making it likely that both the drugs (and by implication the other phenothiazine derivatives used) exert their effects on a specific mechanism. If the excitation of the hypothalamus by promethazine were non-specific it is unlikely that chlorpromazine would prevent release of vasopressin. It seems much more likely (because of the structural similarity of the two drugs) that they both act on the same mechanism and that this mechanism is important for the release of vasopressin because its depression can block hormone release by all the stimuli investigated.

Consideration of the molecular structure of the small number of compounds investigated suggests that the important features of the molecule of the phenothiazine derivatives which determine whether they release or inhibit the release of vasopressin are the length of the side chain, the existence of a branch on the side chain and the size of the groups attached to the terminal nitrogen of the side chain. Thus chlorpromazine, with three carbon atoms in the side chain, no side chain branch and methyl groups on the terminal nitrogen, inhibits release. Trimeprazine, with a three carbon side chain, a branch on the side chain and methyl groups on the terminal nitrogen causes some release of vasopressin and then blocks release by haemorrhage. Ethopropazine with a two carbon side chain, a side branch and ethyl groups on the terminal nitrogen, causes some release and a partial blockade of subsequent release by haemorrhage. Promethazine, however, with a two carbon side chain, a branch on the side chain and methyl groups on the terminal nitrogen of the side-chain causes release of vasopressin and no subsequent blockade of release by haemorrhage. Chlorpromazine *N*-oxide, like its parent molecule, did not release vasopressin and prevented the release of vasopressin by haemorrhage.

The mode of action of the phenothiazine derivatives on vasopressin release is not clear but there seem to be at least two possibilities. None of the acetylcholine antagonists investigated inhibited release in response to the stimulus of severe haemorrhage. This suggests that there may be an alternative pathway for vasopressin release which does not involve the cholinergic step blockade by mecamylamine and compounds A to F during calcium chloride injection and vagal stimulation. (The cholinergic step presumably corresponds to that stimulated by acetylcholine in the experiments of Pickford (1939, 1947), Duke & Pickford (1951) and Duke, Pickford & Watt (1950).) This hypothesis is consistent with the suggestion of Feldberg (1950) that the nerve fibres converging on the supraoptic nucleus are cholinergic, but that the last neurone by which nerve impulses are conveyed to the posterior pituitary is non-cholinergic. If so, this pathway may involve a hitherto unsuspected type of central nervous transmission by a substance which can be mimicked by promethazine and blocked by chlorpromazine.

The other alternative is that the phenothiazine derivatives influence the cholinergic neurones and that promethazine, ethopropazine and trimeprazine act as cholinomimetic

substances and that chlorpromazine and its *N*-oxide act as antagonists of acetylcholine. There are reports that phenothiazine derivatives can affect cholinergic transmission because as Todrick (1954) demonstrated, some have anticholinesterase activity. In addition, several phenothiazine derivatives have been shown to inhibit contraction of skeletal muscles after application of acetylcholine (Jindal & Deshpande, 1961) and electrical activity of the cerebral cortex induced by neostigmine and di-isopropyl-fluorophosphate (White & Westerbeke, 1961). If the oxygen atom which links the carbamate and choline groups in the carbachol molecule is considered to be comparable with the nitrogen atom in the phenothiazine ring of promethazine, a structural similarity between promethazine and carbachol can be seen. This provides a possible chemical basis for the proposed cholinomimetic action of promethazine because carbachol is structurally very closely related to acetylcholine and if injected into the common carotid artery can itself cause some release of vasopressin (Dyball, 1968).




SUMMARY

1. The effects of a number of drugs on vasopressin release by haemorrhage, stimulation of the central end of the severed right vagus nerve and calcium chloride injection were investigated.

2. None of the acetylcholine antagonists used (including ganglion-blocking drugs) inhibited the release of vasopressin by haemorrhage although, with the exception of hexamethonium (which seemed to have no effect on vasopressin release) and pempidine (which inhibited release only by calcium chloride injection), all of them inhibited release by vagal stimulation and calcium chloride injection.

3. Adrenaline and phenoxybenzamine produced some degree of inhibition of vasopressin release but tyramine and noradrenaline were without effect.

4. Chlorpheniramine and reserpine appeared to have very little effect on vasopressin release.

5. Ethanol caused some inhibition of vasopressin release by vagal stimulation and calcium chloride injection but the effects were not consistent. It seemed to have no effect on release by haemorrhage.

6. Chlorpromazine prevented the release of vasopressin by all three stimuli and its *N*-oxide inhibited the release by haemorrhage. Promethazine, ethopropazine and trimeprazine, however, caused a release of vasopressin. Promethazine was the most effective of these drugs but its effect on vasopressin release could be prevented by chlorpromazine.

7. The differences in the action of the different phenothiazine derivatives used are discussed with relation to the differences in their molecular structure.

8. The results described are consistent with two possible interpretations. First, that the pathway for vasopressin release by haemorrhage does not involve a cholinergic step. Second, that the phenothiazine derivatives are acting either as cholinomimetic substances or as antagonists of acetylcholine.

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