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THE EFFECT OF ANTICHOLINESTERASES INJECTED INTO THE SUPRAOPTIC NUCLEI OF CHLORALOSED DOGS ON THE RELEASE OF THE OXYTOCIC FACTOR OF THE POSTERIOR PITUITARY

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In a paper by Duke, Pickford & Watt (1950) experiments are described in which diisopropylfluorophosphate (DFP) injected amongst the supraoptic nerve cells of dogs gave rise, after an immediate and profound inhibition of the rate of urine flow, to a temporary diabetes insipidus lasting from 4 to 19 days, and then an inability lasting 89–139 days to respond by antidiuresis to an intravenous injection of acetylcholine. Thereafter there was an apparently complete return to normality. From these observations, and some earlier ones (Pickford, 1947), it was concluded that acetylcholine was the transmitter at the supraoptic neurones. Since antidiuretic hormone (ADH) always seems to be released in company with the oxytocic factor of the posterior lobe of the pituitary it seemed probable that the oxytocic factor, too, depends for its liberation on a similar cholinergically transmitted stimulus.

In the experiments described in this paper eserine and DFP were injected amongst the supraoptic neurones of anaesthetized dogs and the effect on uterine movements noted. The results showed that spontaneous uterine activity increased following the injection of these two drugs into the supraoptic nuclei, but that it was unaffected by the injection at the same site of small volumes of 0·9% NaCl solution.

METHODS

The first stage of the experiments was to give each animal four doses of 0·5 mg stilboestrol diproprionate in oil (British Drug Houses) subcutaneously on alternate days in order to sensitize the uterus to the action of posterior pituitary oxytocic factor and to induce spontaneous activity. The day after the last stilboestrol injection a preliminary aseptic operation was performed under sodium pento-barbitone anaesthesia to expose the pituitary gland and optic chiasma. Early the following morning when the animal was well and lively it was given half a pint of milk to which water and glucose were added. Two or three hours later anaesthesia was induced either by giving 0·14 g chloralose per kg body weight by mouth in 250–300 ml. water, or by the intravenous injection of 0·1 g chloralose per kg body weight in 0·9% NaCl solution. Two methods were used

for recording uterine movements. In the first, a balloon inserted into the uterus contained water in continuity with that in a small reservoir held 25 cm above the level of the uterus. Changes in water-level in the reservoir caused by uterine contractions were transmitted by air to a 5 ml. volume recorder provided with a stylus writing on a kymograph. The abdomen was closed after insertion of the uterine balloon. In the second method, the upper end of one uterine horn was freed and tied to a thread passing over pulleys to a recording lever. The uterus was prevented from cooling and drying by passing it through a glass tube filled with warm paraffin. The abdomen was closed round the tube. In both methods the lower end of the uterus was fixed by gripping the cervix in volsellum forceps passed up the vagina, the forceps being clamped to a stand. The DFP was made up immediately before use as a part solution–part emulsion in 0·9% NaCl solution and well shaken. The eserine salicylate was also made up immediately before use. The volume of the injections was 0·002 ml. The method of making the injections has already been described (Pickford, 1947).

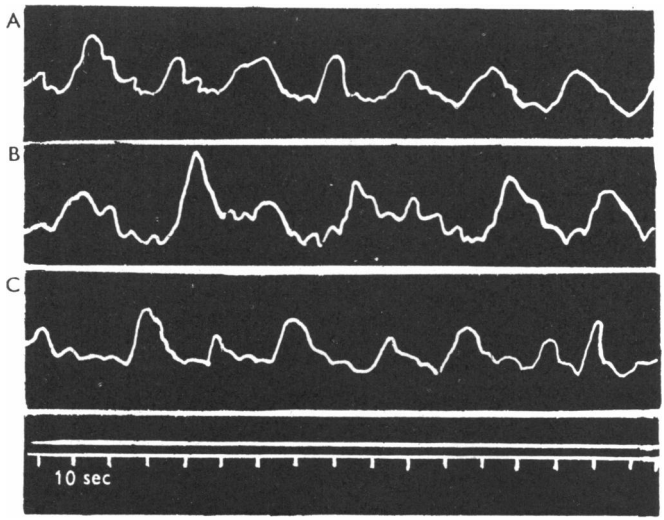

Fig. 1. Record of uterine contractions. A, normal spontaneous movements; B, 30 min after injection of 40μg eserine salicylate into the left supraoptic nucleus; C, 60 min after the injection. Time, 10 sec.

RESULTS

Observations were made on a total of seven dogs. The results were the same whichever system of recording or anaesthesia was used. Following the pre-treatment with stilboestrol all the uteri showed steady spontaneous activity (Figs. 1 A and 2 A).

Effect of 0·9% NaCl solution. The injection of 0·002 ml. 0·9% NaCl solution into the area of the supraoptic nuclei was wholly without effect on the pattern of uterine activity.

Effect of eserine salicylate. On two occasions 40μg eserine salicylate in 0·9% NaCl solution was injected into one or other supraoptic nucleus. In both instances the result was the same. Four to five min after the injection the spontaneous uterine contractions increased in size and remained large for

45–50 min, they then began diminishing until in about an hour after the injection they were once more the normal size (Fig. 1).

Effect of DFP. DFP was used on three occasions in doses of 40, 80 and 100 μg, and each time the result was the same. Four to five min after the injection into the supraoptic nuclei uterine contractions began to increase in size, attained their maximum in about 15 min and remained large for the rest of the experiment. The longest time of observation was 4½ hr after the injection was made.

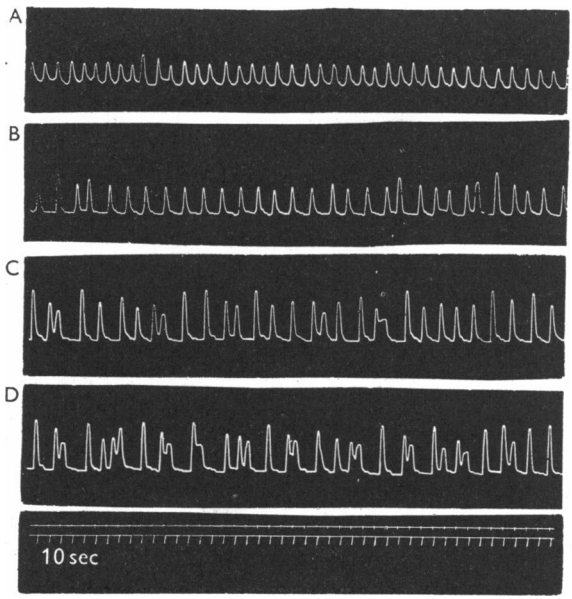

Fig. 2. Record of uterine contractions. A, normal spontaneous movement; B, 4 min after injection of 80μg DFP into left supraoptic nucleus; C, 1½ hr after the injection; D, 4 hr after the injection. Time, 10 sec.

In the two remaining experiments the injection of eserine and DFP was without effect. Histological examination of the hypothalamus showed that the injections had been badly placed, in one instance the tip of the needle had been lateral to one fornix, and in the other it had penetrated to the posterior hypothalamus.

Owing to the impossibility of maintaining the uterus in a steady state of spontaneous activity for several weeks no attempt was made to follow the after-effect of DFP injection on the uterus to see if it paralleled the effect on the excretion of water by the kidney.

DISCUSSION

Injection of eserine or DFP into the supraoptic nuclei of chloralosed dogs led to a rapid increase in size of spontaneous uterine contractions. After the former drug the increased activity lasted for about an hour, after the latter it lasted for the duration of the experiment, i.e. for at least 3–4½ hr. No uterine reaction followed injection of anticholinesterases into two other sites in the hypothalamus. Since the anticholinesterase action of eserine is short-lived and that of DFP prolonged, if the oxytocic factor, like ADH, is liberated by a cholinergic mechanism, then the above results are to be expected. The findings, then, support the view that a cholinergic transmitter to the supraoptic neurones is responsible for the release of the oxytocic, as of the antidiuretic, factor of the posterior lobe. The results are also in agreement with others showing that any stimulus causing the release of one posterior pituitary active substance also causes the simultaneous release of the other (Abrahams & Pickford, 1954; Harris, 1955), but they offer no solution to the problem of how differing proportions of the two factors may be liberated at different times.

SUMMARY

1. Observations were made of the effect on spontaneous uterine contractions of injecting eserine salicylate and DFP into the supraoptic nuclei of chloralosed dogs.

2. Both eserine and DFP caused an increase in size of uterine contractions. The effect of eserine lasted for about an hour, and that of DFP for at least 3–4½ hr.

3. These findings support the view that the oxytocic, like the antidiuretic factor, of the posterior pituitary can be released by cholinergic transmission to the supraoptic neurones.

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