Brit. J. Pharmacol. (1957), **12**, 484.

THE ANTIDIURETIC ACTION OF 5-HYDROXYTRYPTAMINE IN CATS IN RELATION TO THE PRODUCTION OF CERTAIN CHEMOREFLEXES

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(RECEIVED MAY 29, 1957)

In cats lightly anaesthetized with cnloralose, small (5 to 50 μg./kg.) intravenous doses of 5-hydroxytryptamine temporarily reduced the flow of urine into the bladder in addition to causing reflex falls of blood pressure and heart rate and temporary arrest of breathing. Doses of phenyl diguanide and other aryl diguanides, the reflex effects of which on blood pressure and respiratory movement approximately matched those of 5-hydroxytryptamine, had comparatively little antidiuretic effect. Phenyl diguanide, unlike 5-hydroxytryptamine (tested previously under similar conditions), did not have an antidiuretic effect in hydrated mice in doses of 0.05, 1, and 2.5 mg./kg. subcutaneously. It is concluded that the reflex depressor action of 5-hydroxytryptamine plays little part in reducing urinary output.

Several different views have been expressed about the mechanism of the antidiuretic action of 5-hydroxytryptamine (5-HT). Corcoran, Masson, del Greco, and Page (1954) suggested that the antidiuretic effects observed in rats and dogs are, for the most part, brought about indirectly by stimulation of the posterior pituitary due to the pain of the injection and by lowering of blood pressure. Actually, antidiuretic doses of 5-HT in dogs have usually been found to raise rather than lower blood pressure (Abrahams and Pickford, 1956a and b ; Spinnazola and Sherrod, 1955). Del Greco, Masson, and Corcoran (1956) have since modified their views: they have observed that the antidiuretic effect of 5-HT in the rat is dependent on decreased glomerular filtration but largely independent of changes in blood pressure. Although Sala and Castegnaro (1953) attribute the antidiuretic action of 5-HT in the dog principally to an increased reabsorption of water from the renal tubules, other workers (Erspamer, 1954 ; Spinnazola and Sherrod, 1955 ; Abrahams and Pickford, 1956a) have found the antidiuretic effect of 5-HT to be attended by changes in glomerular filtration rate rather than in renal plasma flow. It has been shown that renal blood vessels are constricted by 5-HT (Page, 1952 ; Cerletti, Carpi, and Rothlin, 1955) and that the intact kidney pales when 5-HT is given intravenously (Erspamer, 1954 ; Abrahams and Pickford, 1956a). According to Erspamer and Asero (1952), 5-HT has a specific constrictor action on the afferent bed of the glomerulus.

Abrahams and Pickford (1956a) agree with Erspamer that vasoconstriction is a prerequisite for antidiuresis, but they believe that kidney blood vessels are no more sensitive to 5-HT than those of certain other areas. They noticed that when antidiuresis occurred after 5-HT had been given to dogs it was accompanied by obvious respiratory reactions. This was one of several pieces of evidence which suggested to them " that 5-HT produces antidiuresis mainly by setting up reflexes from the thorax, probably from the pulmonary bed, which, by altering the general blood pressure, affect the kidney."

Since there is a great difference between the cat and the dog as regards the effects of 5-HT on the circulatory and respiratory systems, we thought it of interest to see whether there is any obvious relationship between the antidiuretic activity of 5-HT in the cat and its " reflex " activity in that species. Whereas in the dog the coronary and allied chemoreflexes play little part in the circulatory response, in the lightly anaesthetized cat the chief circulatory effect of 5-HT is an abrupt fall of blood pressure and heart rate brought about reflexly by stimulation of chemoreceptors in the heart and lungs. In the dog 5-HT causes hyperpnoea by stimulating the aortic and carotid bodies,

but in the cat 5-HT causes temporary arrest of breathing by a chemoreflex involving sensory receptors in the lungs (Page, 1953 ; Dawes and Comroe, 1954 ; Kottegoda and Mott, 1955). We wondered whether the antidiuretic activity of 5-HT in the cat would be comparable to that in the dog despite these other differences. As it seemed possible that the hypotension evoked reflexly by 5-HT in the cat might contribute substantially to its antidiuretic action, we have tested in addition to 5-HT such compounds as phenyl diguanide which closely resemble 5-HT in their ability to produce the coronary and certain other chemoreflexes though not in most other respects (Dawes and Mott, 1950 ; Dawes and Comroe, 1954).

METHODS

Cats were anaesthetized with chloralose (usually 70 mg./kg.). The trachea and a saphenous vein were cannulated. Blood pressure was recorded from a carotid artery. Respiratory movements were recorded by a modification of the method of Gaddum (1941). To measure urine flow, the bladder was cannulated after the urethra had been tied and the cannula was connected by a long 2 mm. bore rubber tube to a Condon drop counter, which was placed on the ground 3 ft. below the level of the cat so that there was a slight negative pressure to prevent the urine from remaining in and distending the bladder.

Rabbits were anaesthetized with sodium pentobarbitone. Drug solutions were injected into them by a jugular vein.

The effect of phenyl diguanide on water diuresis in mice was studied by the technique of Waal and Veale (1956) which had been used in earlier experiments with 5-HT. During a 19 hr. period each mouse was given by stomach tube three doses of lukewarm water, each of 5% of its body-weight. Immediately after the third dose the mice were given a subcutaneous injection of 0.01 ml./g. of phenyl diguanide hydrochloride in 0.9% NaCl or of saline alone. To minimize individual variation, which was considerable, comparisons were made of the urinary output of groups of 4 mice. Each group was confined in a small cage above a collecting funnel and measuring cylinder. The amount of urine excreted was measured at 15 min. intervals for 6 hr. A water diuresis was produced in duplicate experiments, each with 8 batches of 4 mice tested on 4 different days. The mice of each batch eventually received 1 control injection of saline and 3 injections of phenyl diguanide hydrochloride, the test solutions of the latter containing 0.005, 0.1, and 0.25 mg./ml. respectively. To eliminate any effect due to tolerance, the treatments were randomized by two independent 4 by 4 latin squares.

The 5-HT used was serotonin creatinine sulphate monohydrate (May and Baker). The aryl diguanides were synthesized in the department.

RESULTS

Experiments on Cats.—Small doses of 5-HT (20 to 50 μg./kg.) decreased urine flow for periods ranging from 30 to 100 sec. in each of 10 female cats weighing 2.5 to 3.2 kg. Definite antidiuretic effects were produced in several of the cats with doses as low as 3 μg./kg. of the salt (almost equal to 1.5 μg./kg. of the base). The antidiuretic effect was often followed by an equally brief diuretic effect (Fig. 1). Long-lasting antidiuretic effects were not obtained even when 30 μg./kg. doses of 5-HT were given every 5 min. for 30 to 60 min. The duration of the antidiuresis obtained with a 30 μg./kg. dose appeared to depend on the initial urine flow ; the greater the rate of flow of urine the more transient was the antidiuretic effect.

In the same range of doses, 5-HT usually caused a temporary fall of blood pressure and arrest of breathing (Fig. 1). The antidiuretic effect

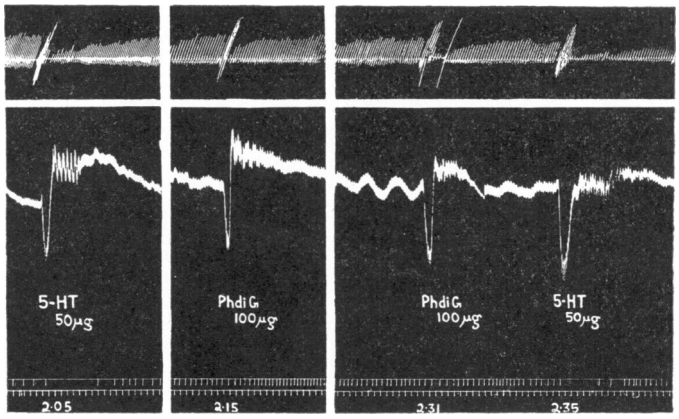

FIG. 1.—Cat. 3.2 kg. female. 210 mg. chloralose. Records from above downwards: respiratory movement, arterial blood pressure, urinary output, time (10 sec.). Note that phenyl diguanide (PhdiG) had comparatively little antidiuretic effect in amounts which had nearly the same effect on blood pressure and respiratory movement as the doses of 5-hydroxytryptamine (5-HT) used.

occurred after the trough of the hypotension. Several lines of evidence indicated that the antidiuretic effect is largely independent of the reflex actions on the circulatory and respiratory systems.

Firstly, with 3 of the cats pressor responses to 5-HT were obtained for a period of the experiment. Despite this reversal (for no ascertainable reason) of the normal effect of 5-HT on blood pressure, an antidiuretic effect was still produced (Fig. 2). Antidiuretic effects were sometimes obtained also in the absence of the respiratory reflex. Conversely, the usual reflex effects of 5-HT were obtained in a few instances in the absence of an antidiuretic effect.

Secondly, the reflex effects of 5-HT on blood pressure and respiratory movement could be matched fairly closely by injecting phenyl diguanide in suitable doses (Fig. 1). The 5-HT was about twice as active as phenyl diguanide on a molar dosage basis. In experiments performed on 8 of the cats, the antidiuretic effect of 5-HT was invariably greater than that of phenyl diguanide given in equidepressor amounts. In fact, doses of phenyl diguanide sufficient to lower blood pressure by 50 mm. Hg or more often had little or no antidiuretic effect. A brief diuretic response to phenyl diguanide was noted sometimes, especially when there was a secondary rise of blood pressure after the hypotension. With the *o*-, *m*-, and *p*-chlorophenyl diguanides and the *o*-, *m*-, and *p*-tolyl diguanides, as with phenyl diguanide, the antidiuretic effects (if any) were substantially less than those produced by 5-HT in equidepressor doses. In one experiment, acetylcholine and amyl nitrite were given in doses which lowered blood pressure to approximately the same extent as large doses of 5-HT and phenyl diguanide. All 4 drugs had a transient antidiuretic effect (Fig. 3). The antidiuretic effects of acetylcholine and amyl nitrite were comparable with those of phenyl diguanide, possibly because the former drugs produced more persistent falls of blood pressure.

Thirdly, vagotomy, which blocks the reflex actions of 5-HT and phenyl diguanide, did not abolish the antidiuretic action of 5-HT in 4 out of 6 cats. The effect of vagotomy on the antidiuretic action of 5-HT was difficult to assess, however, because after vagotomy the blood pressure usually rose, the rate of urine flow increased, and the response to 5-HT was usually pressor. The greater flow of urine due to increased blood pressure might explain why the antidiuretic effect of 5-HT was abolished by vagotomy in 2 of the cats and was considerably reduced by it in the others.

In another 3 experiments on cats, a large incision was made in the abdomen and part of the bowel pulled through the opening to permit direct observation of the right kidney and the ureter. Even when 5-HT was given in doses as large as 50 μg./kg., it did not cause paling of the kidney, nor did it throw the ureter into obvious spasm.

Experiments on Rabbits.—When the same kind of experiment was performed on 3 rabbits, the effect of 5-HT was much more pronounced. The paling of the kidney after large doses (20 to 50 μg./kg.) was striking and persistent. In 2 of the rabbits, the intravenous injection of 5-HT caused a rigor, more vigorous peristalsis, defaecation, and urination.

Experiments on Mice.—To see whether phenyl diguanide has an antidiuretic effect in mice, sub-

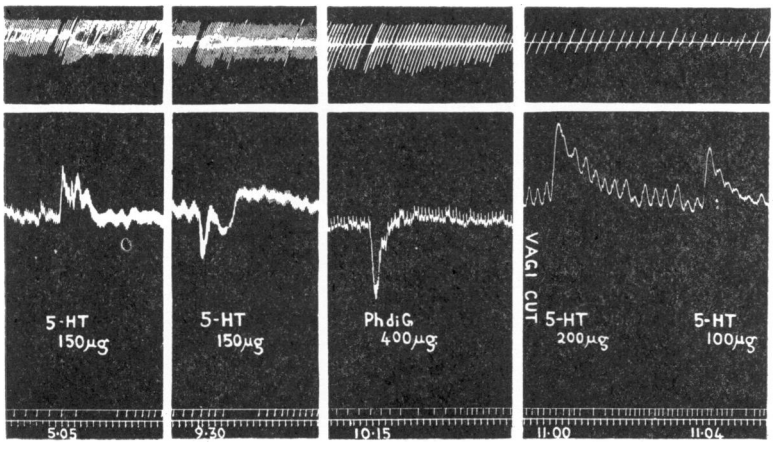

FIG. 2.—Cat. 3 kg. female. 210 mg. chloralose. Records from above downwards: respiratory movement, arterial blood pressure, urinary output, time (10 sec.). Note that 5-HT had a transient antidiuretic effect even when it produced a rise of blood pressure and after vagotomy. Phenyl diguanide (PhdiG) had a smaller antidiuretic effect despite the greater fall of blood pressure it produced.

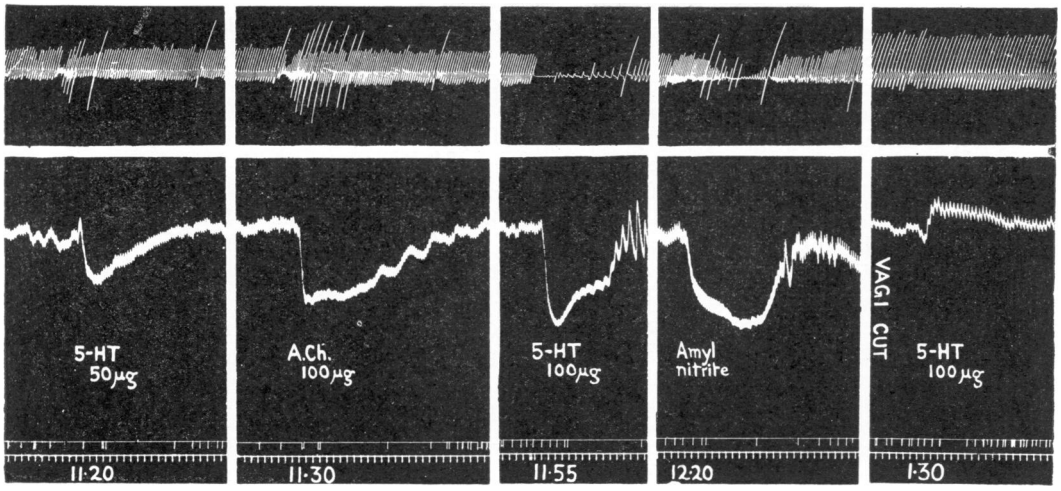

FIG. 3.—Cat. 3 kg. female. 200 mg. chloralose. Records from above downwards: respiratory movement, arterial blood pressure, urinary output, time (10 sec.). Note that 5-HT had a transient antidiuretic effect even when it produces a pressor response after vagotomy, and that acetylcholine (A.Ch.) and amyl nitrite (0.01 ml.) also have an antidiuretic effect when given in amounts producing substantial falls of blood pressure.

cutaneous doses of 0.05, 1, and 2.5 mg./kg. were given to hydrated mice. The amounts of urine excreted each ½ hr. up to 6 hr. after treatment were taken as the results for an analysis of variance. This analysis showed that neither the duplication nor the order of treatment had a significant effect. Nor was the average excretion rate, and hence the volume of fluid excreted over the total 6 hr. period, significantly altered by phenyl diguanide in the 3 concentrations tried. However, the two higher concentrations had a definite diuretic effect in that the peak diuresis at 1.5 hr. was significantly ($P<0.001$) higher than the peak diuresis for the controls, which also occurred 1½ hr. after the last dose of water.

DISCUSSION

In the cat small intravenous doses of 5-HT cause sharp falls of blood pressure in addition to decreasing urinary output for brief periods. Hypotension *per se* can have an antidiuretic effect. However, the antidiuretic effects which we observed cannot be attributed wholly or even mainly to the hypotensive action of 5-HT, because doses of phenyl diguanide which caused practically identical changes in blood pressure had little or even no antidiuretic effect. In the mouse, under conditions in which 5-HT shows strong antidiuretic activity (Waal and Veale, 1956), phenyl diguanide tended to increase rather than decrease urinary output.

The antidiuretic effects which 5-HT has been reported to produce in such animals as the rat and mouse are long-lasting (Erspamer, 1954; Waal and Veale, 1956), quite unlike those we have obtained in the cat. Now Abrahams and Pickford (1956b) have shown that small intravenous doses of 5-HT (5 to 20 μg.) exert such a powerful action on the ureteric muscle of the dog that the passage of fluid through the lumen of the ureter can be completely prevented for a minute or two and slowed for a further period of 1 to 2 min. Several findings support the idea that the antidiuretic effect of 5-HT in cats is due to ureteral spasm, such as the transience of the effect, the secondary diuretic effect of 5-HT in some cats (for urine held back by ureteral spasm would accumulate in the renal pelvis until the muscle relaxed, when a comparatively large volume at high pressure would be delivered in a short time), and the shorter duration of the antidiuretic effect with high rates of urine flow (for the greater the urine flow the sooner would a pressure be reached sufficient to force the ureter open). Nevertheless, when a length of ureter was observed directly, no noteworthy effect on tone could be detected after the injection of 5-HT.

In contrast to the cat kidney, the rabbit kidney became much paler when the animal was given a large intravenous dose of 5-HT. Moreover, the injection of 5-HT produced a number of other striking effects in the rabbit, due mainly it appeared to a sudden widespread increase in muscle tone.

Although the experiments on rabbits were not strictly comparable with those on cats in that a different anaesthetic was used and part of the alimentary tract was excised to give a good view of the kidney, it seems likely that the very different results obtained in the two series of experiments are due to species differences. Many of these have already been noted in studies with 5-HT ; some are mentioned earlier in this paper. Because of the likelihood of species differences, we hesitate to attribute the antidiuretic effects of 5-HT in the cat to either ureteral spasm or constriction of renal blood vessels, though we do not doubt the importance of these mechanisms in certain other species.

It is a pleasure to acknowledge our indebtedness to William R. Warner & Co. and to the Medical Research Council of New Zealand for financial assistance.

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