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SOME CENTRAL ACTIONS OF 5-HYDROXYTRYPTAMINE AND VARIOUS ANTAGONISTS

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The pharmacological analysis of extracts of the central nervous system led to the conclusion that 5-hydroxytryptamine (HT) was present in such extracts and was distributed in a way that suggested it might play a part in the physiology of certain nervous centres and particularly those near the 3rd and 4th ventricles (Twarog and Page, 1953; Amin, Crawford, and Gaddum, 1954). A study of the distribution of the enzyme 5-hydrotryptophan decarboxylase, which forms HT, strengthened this conclusion (Gaddum and Giarman, 1956). The discovery that lysergic acid diethylamide (LSD) was a powerful and specific antagonist for HT (Gaddum, 1953; Gaddum and Hameed, 1954) suggested that the actions of this substance on the brain might be due to this antagonism (Gaddum, 1954; Woolley and Shaw, 1954). These actions were described by Stoll (1947) and have received much attention in recent years. The most obvious effects of small doses in man involve changes in personality and perception which have been compared with the symptoms of schizophrenia. The experiments described here were undertaken with the object of discovering whether a known central action of HT was in fact antagonized by LSD. Central actions of HT in cats, uncomplicated by general vascular effects, can only be observed by giving it intrathecally or into the cerebral ventricles. The effect of injecting HT into the lateral ventricle of a conscious cat had been described by Feldberg and Sherwood (1954). It was thought that the effects might be specifically antagonized by small doses of LSD.

METHODS

Metal cannulae were screwed into the skulls of cats anaesthetized with pentobarbitone, as described by Feldberg and Sherwood (1953), and injections were made through these cannulae into one lateral ventrical during the succeeding weeks or months. The effects of the injections were recorded merely by observing the behaviour of the cats.

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (Abbott Laboratories), lysergic acid diethylamide tartrate (Sandoz, Ltd.), reserpine solution ("Serpasil," Ciba Laboratories), ergometrine maleate, 2-bromo-lysergic acid diethylamide tartrate ("Bol 148," Sandoz, Ltd.), 1 : 2-dimethyl 3-ethyl 5-dimethylamino-indole ("methylmedmain"), 5-benzyloxygramine, morphine HCl, methadone HCl, amphetamine sulphate, methylamphetamine HCl (Methedrine, Burroughs Wellcome & Co.).

Most drugs were dissolved in sterile 0.9% NaCl solution and injected in a volume not exceeding 0.2 ml. Methylmedmain was dissolved in the equivalent amount of 0.15N-HCl to make an approximately 3% solution of the base. Benzyloxygramine, the hydrochloride of which is not very soluble, was heated with the equivalent amount of dilute HCl and a 1% solution (in terms of the base) prepared by the addition of 0.9% NaCl solution. The mixture was slightly cloudy.

Doses of HT are given in terms of the base, of the other salts as weight of the salt.

RESULTS

HT.—The observations of Feldberg and Sherwood (1954) on the effects of HT on cats were in general confirmed. Doses of 0.1–0.6 mg. caused a curious lethargy during which the cats, which had been energetic and enterprising, became hesitant and retiring. They lost initiative and instead of wishing to run about they appeared anxious to return to their cages. There was diminished muscular tone, so that the body was apt to sag, but the cat could be roused. The respiration was very rapid during the first 30 min. after the injection.

When no other injections were made, these effects lasted at least 6 hr., but the cats were normal the next day. The effects observed by Feldberg and Sherwood apparently did not last so long as this. The reason for this difference in the duration of the effect is not known.

Reserpine.—The depressant actions of this drug on the central nervous system have been widely studied (Conferences, 1954, 1955). According to

Shore, Silver and Brodie (1955), and Pletscher, Silver and Brodie (1955), reserpine releases HT in the body, causing depletion of tissue HT and the appearance of a metabolite of HT in the urine. The depletion of HT in brain extracts has also been observed by Paasonen and Vogt (1956). It is therefore suggested that the effects of reserpine are due either to the disappearance of HT from the tissues, or to the flooding of the body with excessive amounts of this substance. The observation of Shore, Silver, and Brodie (1955) that reserpine and HT both increase the effects of hypnotics when injected intraperitoneally in mice is in favour of the second of these possibilities, and the results obtained with cats point to the same conclusion, since both drugs have a depressant action.

The intraventricular injection of 10 μg. reserpine had no obvious effect during 3½ hr. observation. In most of the experiments with this drug, however, it was given intraperitoneally in a dose of 0.25–0.5 mg./kg. in the afternoon or evening. This generally caused diarrhoea in the first few hours and the cats gradually became depressed; the next morning the sedation was marked, the pupils narrow, the nictitating membrane relaxed and the eyes nearly shut; the cat sat quietly in its cage and walked clumsily when disturbed. The inertia produced by reserpine was not unlike that due to HT, though the effects on the pupil and nictitating membranes were not observed with HT, and the ataxia was more pronounced with reserpine. It is possible that the mode of administration is responsible for some of these differences. Drugs given systemically are likely to reach sites inaccessible to drugs injected into the cerebral ventricles.

In one cat 0.3 mg. HT was injected intraventricularly at an interval of 16 hr. after reserpine. The only apparent result of this second injection was that the cat became even more lethargic.

LSD.—The injection of 1–20 μg. into the cerebral ventricle had no obvious effect. A dose of 1 μg./kg., given by the mouth to man, has profound subjective effects and it might therefore be expected that the injection of 20 μg. into the ventricle would have had a marked action on the cat. It is, of course, impossible to know what subjective effects may have been produced, but the cat's behaviour was apparently unaltered after this dose.

The injection of 0.2 mg. LSD intraventricularly caused minor symptoms such as salivation, and the injection of 0.8 mg. caused "sham rage." Cats which had been quiet and friendly became for a time unreasonable and intolerant. They hissed fiercely and bared their teeth when approached, and seemed to resent even the smallest movement or remark. These effects appeared in about 3 min. and lasted about 45 min.

The subcutaneous injection of 3 mg. LSD in one of these cats caused symptoms of the same kind lasting about 2 hr.

When 0.2 mg. HT and 0.8 mg. LSD were injected simultaneously, the effect of the LSD appeared first and after about 45 min. the cat appeared nearly normal. About 4 hr. after the injection it became lethargic, presumably owing to the continued action of the HT.

When 0.3 mg. HT was followed by 0.8 mg. of LSD 45 min. later the lethargy due to the HT was interrupted by the LSD and returned 3 hr. later. While the LSD was acting, the cat was as excited, intolerant and restless as if the drug had been given alone. During this phase its muscular strength appeared quite normal.

In an experiment with another cat, which was normally energetic, 0.6 mg. HT caused very marked apathy; the injection of 0.8 mg. LSD 45 min. later overcame the apathy and caused great excitement.

The effect of reserpine was also antagonized by LSD. A cat received reserpine (0.25 mg./kg. intraperitoneally) and 20 hr. later LSD (2 mg. subcutaneously). The reserpine had its usual depressant effect and the eyes were nearly shut, the pupils contracted, and the nictitating membrane relaxed. The LSD roused the cat in about 20 min. and made it restless and angry, but it was clumsy and did not move much. All the eye symptoms had disappeared—the eyes were open, the pupils dilated and the nictitating membranes retracted. This effect lasted about 1½ hr., after which the typical effects of reserpine returned—the cat became inert and the eye symptoms returned.

These experiments left no doubt that the apathy due to HT or reserpine temporarily disappeared under the action of LSD.

Ergometrine.—The molecule of ergometrine is very similar to that of LSD, but this drug is less active as an antagonist of the action of HT on rat's uterus (Gaddum and Hameed, 1954). Recent experiments (Blair, personal communication, 1955) indicate that its activity on the rat's uterus as an antagonist of HT is about 1/10 that of LSD.

Ergometrine (2–2.5 mg./kg. intravenously) is known to cause sham rage in cats (Brown and Dale, 1935).

When 2 mg. ergometrine maleate was injected intraventricularly the effect was similar to that of LSD, though not the same. After 10 min. the cat was salivating but sitting immobile. Its pupils then widened and after 25 min. it became ataxic. After about 40 min. it resented being approached, but

appeared frightened rather than angry. The cat was more ataxic and less active than it had been after LSD. This effect lasted for over 6 hr. The injection of 0.4 mg. had similar but smaller effects.

In another experiment, 0.3 mg. HT was followed by 1.5 mg. of ergometrine maleate 28 min. later, both drugs being injected through the cannula. The depressant effect of the HT was antagonized by the ergometrine. The cat which had become lethargic under the influence of HT became restless and apprehensive with dilated pupils about 20 min. after the second injection and remained in this condition for over 2 hr. It was somewhat ataxic and clumsy and not very active, but in other respects the effect of ergometrine was not widely different from that of LSD.

Brom LSD ((+)-2-bromo-lysergic acid diethylamide tartrate).—This substance acts like LSD as an antagonist of HT on rat's uterus, but does not act like LSD on the human brain. These facts provide evidence against the theory that the action of LSD on the human brain is due to this antagonism (Cerletti and Rothlin, 1955).

When a dose of 1 mg. of Brom LSD was injected into a cat's cerebral ventricle, it entirely failed to cause an exhibition of sham rage such as was regularly caused in the same cat by an equivalent dose of LSD (0.8 mg.).

In another experiment, 1 mg. of Brom LSD was injected 1 hr. after an injection of 0.3 mg. HT. No effect was seen except for salivation, but this cat had acquired the habit of salivating in response to all injections. An injection of 0.8 mg. LSD 1 hr. and 25 min. after the Brom LSD caused typical sham rage with dilatation of the pupils, hissing and considerable motor activity.

These results show that Brom LSD does not produce the sham rage and alertness seen after equivalent doses of LSD and that it does not prevent these effects being produced by a subsequent injection of LSD.

Methylmedmain (1 : 2 - dimethyl 3 - ethyl 5 - dimethylamino-indole).—This compound has been found to antagonize some of the peripheral actions of HT (Shaw and Woolley, 1954), and an attempt was therefore made to discover whether it also antagonizes the central action of HT in these cats. The dose was a large one. An isotonic solution of methylmedmain hydrochloride containing 6.2 mg. of the base was injected intraventricularly. Salivation and panting occurred in a few minutes followed, an hour after the injection, by inertia and crouching similar to that produced by HT itself. The action of this drug was quite different from that of LSD. It was clear that central antagonism to HT could not be demonstrated by this technique and no further injections were made.

5-Benzyloxygramine.—This was the most active of a series of new synthetic indole compounds studied by Gaddum, Hameed, Hathway, and Stephens (1954). The intraventricular dose was 2.1 mg. of the base dissolved with HCl in 0.2 ml. of saline; larger amounts could not be dissolved in a suitable volume of fluid. Salivation and a great acceleration of respiration began 10 min. after the injection and persisted for 2 hr. The cat was rather clumsy, but fairly alert and active. A dose of 0.3 mg. HT was then given and produced its usual effect. The injection of as much 5-benzyloxygramine as is practicable thus had no specific effect on the cat's behaviour and did not prevent the action of HT given later.

Morphine.—It is well known that morphine generally causes excitement in cats and depression in dogs. There is some evidence that it antagonizes the action of HT on peripheral tissues (Kosterlitz and Robinson, 1955).

A dose of 2 mg. was injected through the cannula. After 5 min. the cat salivated and defaecated. Its pupils dilated; it ran round the room and appeared agitated and angry. The effect lasted about 4 hr. and had nearly disappeared after 6 hr.

On another day 0.3 mg. HT was injected and the normal depressant effect observed for 44 min. Morphine HCl (1 mg.) was then injected through the cannula and the cat was immediately aroused. After 30 sec. it jumped into the cage with its ears cocked. Within 10 min. it was excited, as it had been after morphine alone. In another cat the effect of HT was reversed in much the same way by the subcutaneous injection of 3 mg. morphine.

In the experiment described above, when reserpine had been injected intraperitoneally and HT through the cannula, an injection of morphine (1 mg.) was given, also through the cannula, 50 min. after the injection of HT. The effect of this injection came on more slowly than those of previous injections of morphine, but after 17 min. it was apparent that the morphine had antagonized most of the effects of both the other drugs. The pupils had partially dilated and the eyes were half open, only the nictitating membranes remained relaxed. The cat now took an increased interest in its surroundings and resisted when picked up. After 45–60 min. the effects of morphine were fully developed and the cat salivated and was excited and unmanageable.

These experiments showed that morphine had an action not unlike that of LSD.

Methadone.—The central effects of this synthetic analgesic are similar to those of morphine. It causes excitement in cats. The injection of 10 mg. methadone subcutaneously caused restless excitement with dilated pupils increasing gradually during the first 1 hr. and lasting about 3 hr. When this same dose was injected at a time when the cat was lethargic following the injection of 0.3 mg. HT intraventricularly, the lethargy disappeared and the cat became excited in much the same way as without HT.

In another cat reserpine (0.25 mg./kg.) was injected intraperitoneally. The next morning, 20 hr. after the injection, this cat was inert with its eyes nearly shut, its pupils contracted and its nictitating membranes relaxed. Methadone (10.5 mg.) was then injected subcutaneously. About an hour later this cat roused itself and rushed about clumsily. The pupils were now widely dilated, but the nictitating membranes were still relaxed.

Amphetamine is known as a central stimulant. It inhibits amine oxidase *in vitro* and it is thought that some of its actions *in vivo* may be due to this effect. The effect of the intraventricular injection of this drug was unexpected. When 0.5 mg. was injected by this route the cat became lethargic for 3–4 hr., after first vomiting. The rate of respiration rose from 37 to 200 during the first 20 min. and then fell to 47 during the next 30 min.

In another experiment, 0.3 mg. HT was injected intraventricularly and followed by 0.1 mg. amphetamine sulphate 30 min. later by the same route. As this second injection did not seem to have affected the normal response to HT, it was followed by the injection of 0.5 mg. amphetamine sulphate 84 min. later. The cat now vomited and urinated and then became more inert than ever. The amphetamine seemed to have intensified the normal depressant action of the HT.

An experiment was then performed in which 0.3 mg. HT was injected into the ventricle and amphetamine sulphate (15 mg./kg.) given subcutaneously 75 min. later. Pupillary dilatation was obvious in a few minutes, and during the course of the next hour the cat became alert, moving its head around a great deal as if searching for something, and running away when approached. Its muscular tone was improved though, except for the incessant head movement, it kept in one place unless stirred. The alertness and searching movements contrasted sharply with the inertia seen after HT alone or after HT and intraventricular amphetamine. The cat remained in the same state for several hours, becoming gradually fiercer and hissing when approached. The effect of amphetamine given subcutaneously to normal cats follows the same pattern and time course. These results show clearly that, in contrast to morphine and LSD which act similarly when given by either route, amphetamine acts differently when injected into the cerebral ventricles and subcutaneously.

Methylamphetamine (Methedrine) and LSD.—The actions of methylamphetamine resemble those of amphetamine.

A few observations were made of the actions of LSD and methylamphetamine in man. One of us took, on four occasions, 30–86 μg. LSD by the mouth and experienced some of the known effects of this drug, such as a feeling of irresponsibility and euphoria, increased awareness of shapes and colours, shimmering of peripheral vision, and a dreamy feeling that sensations did not represent real objects. These subjective effects were accompanied by apparent intoxication and a loss of the power of concentration. Groups of 8 digits were read out from a table of random numbers and the subject tried to repeat them immediately. The normal average score in this test was nearly 100%, but under the action of LSD the score fell to a low figure and fatigue developed rapidly. There was no obvious incoordination, but some loss of the power of reproducing a simple drawing. These effects reached a maximum in about 1½ hr. and then gradually disappeared during the next 3–4 hr.

It was thought that if these effects were due to the inhibition of physiological HT they might be overcome by the administration of methylamphetamine. This drug might be expected to increase the concentration of HT in the brain by inhibiting amine oxidase, and the increased amount of HT might overcome the effect of LSD. This line of reasoning involved various untested hypotheses, but at least it had the virtue of suggesting an experiment which could easily be carried out.

After 3 weeks without drugs 10 mg. methylamphetamine hydrochloride was swallowed at the same time as 86 μg. of LSD. In this experiment there were no obvious signs of intoxication and the ability to repeat random numbers was not impaired. On the other hand, LSD produced the same subjective sensations in this experiment as it did in the other experiments. The conclusion was reached that methylamphetamine had inhibited the objective signs of intoxication by LSD, but had not altered its subjective effects. Self-control was improved, but this is a known effect of methylamphetamine and there is no special reason to believe that it depends on the interaction of methylamphetamine and HT.

Discussion

The first object of these experiments was to demonstrate antagonism between the central effects of HT and LSD, and this was done. The effect of HT was antagonized by LSD, but there was no evidence that the effect of LSD was antagonized by HT. When LSD was given first its effect was so brief that there was no time for the HT to act. When HT was given either before the LSD or at the same time, it caused no clear change in the response to LSD. These results raise doubts about the theory that the central stimulant action of LSD is due to interference with natural mechanisms mediated by HT, and these doubts are strengthened by the results obtained with other drugs.

Experiments with morphine, methadone, ergometrine and subcutaneous amphetamine showed that, although the effects of these drugs differed in detail, they all had the effect of rousing cats from lethargy due to HT. Morphine and methadone also antagonized the main effects of reserpine. All these drugs have in common that they cause sympathetic discharge and the antagonism they exert may come in the category of "arousal" by sympathetic stimulation (Bonvallet, Dell, and Hiebel, 1954).

Brom LSD, methylmedmain and 5-benzyloxygramine all antagonize the effect of HT on the rat's uterus, but they did not rouse cats and there was no evidence that they prevented the central action of HT injected into the ventricle.

These results suggest that the antagonisms studied in these experiments are unspecific and have no relation to the antagonism between HT and LSD in their effects on peripheral organs. The only actions observed, however, were the gross effects of large doses, and the results do not exclude the possibility that some of the central effects of small doses of LSD are due to interference with natural mechanisms mediated by HT. The results of the experiments on man suggest that the central actions of LSD are complex and that antagonists may act on some effects and not on others.

Shore, Silver, and Brodie (1955) observed a central antagonism of LSD to the potentiating action of reserpine on the sleeping time of anaesthetized mice. The dose of LSD (10 mg./kg.) was even greater than that used in the present experiments on cats, and the possibility of a non-specific antagonism might also have to be considered in this work.

It must be remembered that there is no evidence that the effect of intraventricular HT is exerted directly on neurones and not mediated through a constriction of cerebral blood vessels in regions near the ventricles. The depression produced by injecting large doses of adrenaline into the ventricles (Feldberg and Sherwood, 1954) bears much resemblance to the effect of intraventricular HT, and vascular effects may play some part at least in the responses to both drugs when they are administered by this route.

Summary

1. The depressant action of HT on the brains of cats is antagonized by LSD, ergometrine, morphine, methadone and amphetamine, but not by 2-bromo LSD, 5-benzyloxygramine or methylmedmain. The sedation produced by reserpine is antagonized by LSD, morphine and methadone.

2. These antagonisms are probably not related to the specific antagonism between HT and LSD on peripheral tissues.

3. Methylamphetamine inhibits some but not all the central actions of LSD in man.

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