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THE PYRETOGENIC EFFECT OF 5-HYDROXYTRYPTOPHAN AND ITS COMPARISON WITH THAT OF LSD[1]

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One of the reported properties of lysergic acid diethylamide (LSD) is its anti-serotonin action (Gaddum, 1953). Because of this, serotonin (5-hydroxytryptamine) often has been implicated in the central actions of LSD, although no definite proof for this has been presented. Recently several studies (Chessin *et al.*, 1956; Udenfriend *et al.*, 1957 a, b) have indicated that the administration of rather large quantities of the serotonin precursor 5-hydroxytryptophan (5HTP) produced central nervous system excitation. Serotonin does not penetrate the blood-brain barrier in very large amounts (Udenfriend *et al.*, 1957b) but the amino acid, on the other hand, reaches the brain cells without difficulty where it is converted to serotonin. Since 5HTP is rapidly decarboxylated to form serotonin, its central actions are presumed to be due to the formed serotonin, rather than to the amino acid. In each instance these authors believed that there was a close resemblance between the 5HTP and LSD responses. Since there seems to be a similarity in the pharmacological activities as well as in the chemical structures of these two substances, the present experiments were undertaken to investigate and compare the pyretogenic effects of 5HTP and LSD, since the pyretogenic action of lysergic acid diethylamide (LSD) in the rabbit is one of its most sensitive and consistent actions (Horita and Dille, 1954, 1955; Cerletti, 1955).

METHODS AND MATERIALS. Rabbits weighing 1.7 to 2.7 kgm. were used for these experiments. Animals weighing over 3.0 kgm. showed poorer response to the pyretogenic agents. All injections of drugs were made by the intravenous route except in the case of iproniazid which was given subcutaneously. Iproniazid served as the amine oxidase inhibitor decreasing the rapid breakdown of the serotonin formed in the brain. LSD was given subcutaneously during the production of tolerance. Rectal temperatures were measured with a clinical thermometer. They were taken at one-half to one hour intervals for periods of 5 to 7 hours. Room temperature was held constant at 24 ± 1°C.

Tolerance to LSD was induced in the rabbits by daily subcutaneous injections of progressively increasing doses of the drug. The following sequence was used: 1st day, 100 microgm. per kgm.; 2nd day, 200 microgm. per kgm., 3rd day, 400 microgm. per kgm.; 4th day, 700 microgm. per kgm.; 5th day, 1000 microgm. per kgm.; 6th day, 1500 microgm. per kgm.; 7th day 2000 microgm. per kgm. The experiments on cross-tolerance were performed on these animals on the eighth day.

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When iproniazid was used as the amine oxidase inhibitor the 5HTP was given one and one-half hours after the iproniazid. In the BOL-5HTP experiments, 2-brom lysergic acid diethylamide (BOL-148) was given 30 minutes before the injection of the pyretogenic substance.

RESULTS. *The pyretogenic action of 5HTP.* The results of the administration of graded doses of 5HTP to normal rabbits is shown in figure 1. Each curve represents the mean rectal temperatures of from 3 to 6 rabbits. At 25 mgm. per kgm. 5HTP showed little effect on the rectal temperature. Increasing this dose to 50 mgm. per kgm. resulted in a rise of some 1.0 to 1.5°C. with a duration of 3 to 4 hours. If the dose was further increased to 75 and 100 mgm. per kgm., the rise in temperature was marked and rapid, the animals showing temperatures as high as 43 and 44°C. within a period of 30 to 60 minutes after the administration of the amino acid. At the higher dosage ranges the animals frequently died, always succumbing at the peak of the hyperthermic response. In addition to the temperature effects the central nervous system excitation with its characteristic symptoms as described by Udenfriend *et al.* (1957a) have been observed.

In the animals pretreated with 40 mgm. per kgm. of iproniazid a marked

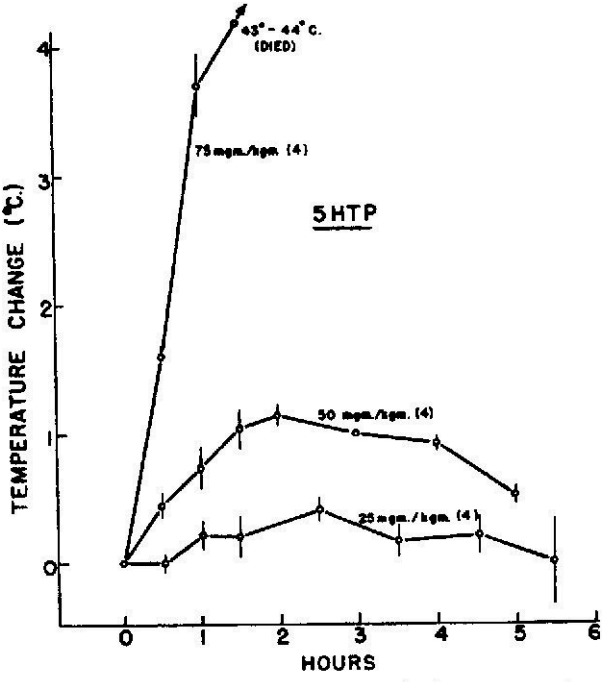

FIG. 1. The pyretogenic effect of graded doses of 5-hydroxytryptophan on the rectal temperature of rabbits.

Each curve represents the mean temperature changes of four rabbits. Vertical lines indicate the standard error of the mean temperature responses. Figures in brackets indicate number of animals used at each dose level.

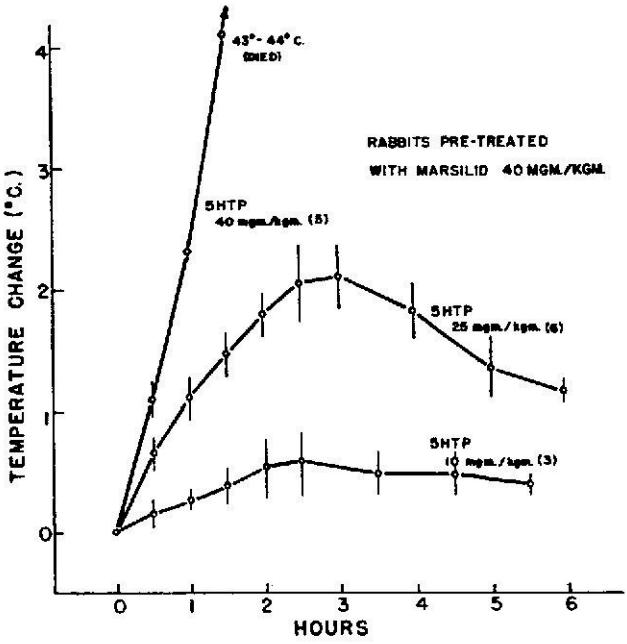

FIG. 2. The effect of graded doses of 5-hydroxytryptophan on the rectal temperature of rabbits pretreated with 40 mgm. per kgm. of iproniazid.

The iproniazid was given 1½ hours prior to the injection of the 5-hydroxytryptophan. Each curve represents the mean rectal temperature changes of from 3 to 6 rabbits. Vertical lines indicate the standard error of the mean temp changes. Figures in brackets indicate the number of animals used at each dose level.

potentiation of the pyretogenic response to 5HTP was seen. Doses of the amino acid which were inactive in producing hyperthermia in normal rabbits now showed considerable activity after iproniazid as shown in figure 2. Following iproniazid, 40 mgm. per kgm. of 5HTP produced temperature increases of 3 to 4°C. In these animals this dose was consistently lethal, the animals always dying at the peak of the temperature response. In animals pretreated with doses of 100 mgm. per kgm. of iproniazid, the dose of 5HTP necessary to produce the pyretogenic effect was reduced to as low as 5 to 10 mgm. per kgm. and lethal effects were seen with 20 mgm. per kgm.

Antagonism of BOL to LSD and 5HTP. The 2-bromo analogue of LSD, BOL-148 possesses none of the psychic actions of LSD in man or experimental animals (Cerletti and Rothlin, 1955). Large amounts of BOL may be given with very little observable effect. Doses of 1.0 mgm. per kgm. show no effect on the rectal temperature of the rabbit. If BOL is administered in this dose 30 minutes before 50 to 100 microgm. per kgm. of LSD the pyretogenic action of the latter drug is markedly attenuated. In the same manner a lethal dose of 5HTP administered to rabbits pretreated with BOL no longer exhibited lethality and its pyretogenic action was greatly diminished. The antagonism of the temperature effect of

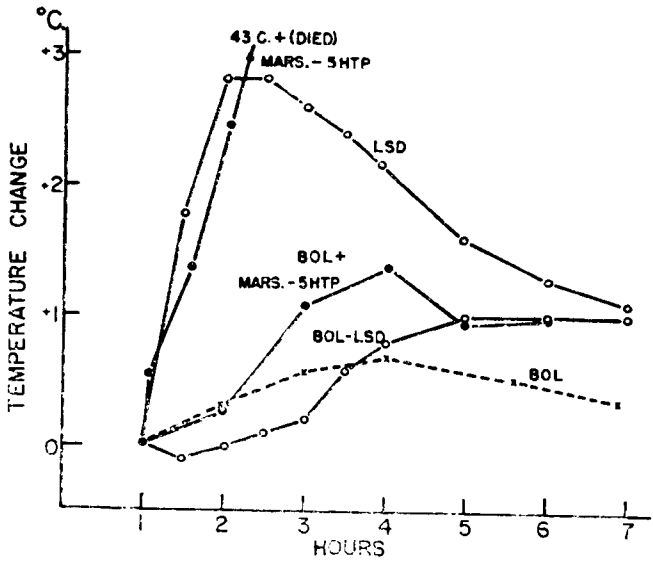

FIG. 3. The effect of BOL on the pyretogenic effects of LSD and 5-hydroxytryptophan. BOL was administered 30 minutes prior to the injections of the pyretogenic agents. The BOL control is represented as a broken line. The doses of drugs used in these experiments were as follows: Iproniazid 40 mgm/kgm.; LSD, 100 microgm/kgm.; 5-HTP 40 mgm/kgm., and BOL, 1.0 mgm/kgm. This graph represents the results from one of four similar experiments. Each curve represents a single animal.

5HTP by BOL was not complete, but a definite reduction is present as shown in figure 3. These experiments therefore demonstrate that the LSD as well as the 5HTP pyretogenic actions are antagonized by large doses of BOL.

Cross tolerance between LSD and 5HTP. The repeated administration of LSD over a period of days produces tolerance to the behavioral and pyretogenic responses (Gogerty and Dille, 1956). With the development of the tolerance it is possible to administer progressively larger amounts of the drug without producing death. In order to determine whether a cross tolerance between LSD and 5HTP might occur, rabbits were made tolerant to LSD by the daily injections of progressively increasing doses of LSD for seven days. By the seventh day a dose of 2.0 mgm. per kgm. of LSD could be given to the animals without lethal effects. When such a dose was given to control animals they promptly showed the marked hyperthermia and death occurred within 30 to 60 minutes. After tolerance was established the animals were treated with a dose of iproniazid and 5HTP which normally produced a non-lethal rise in temperature of some 2 to 2.5°C. A comparison of the responses to 5HTP injections between normal and LSD-tolerant rabbits is seen in figure 4. Whereas the normals show the typical rise, the LSD-tolerant rabbits exhibited only a small increase in temperature, indicating that a cross tolerance phenomenon might be the mechanism of this inhibited response. Increasing the 5HTP doses to normally lethal levels caused the pyretogenic response to reappear. These responses were much less than those in control animals. The cross tolerance between the LSD and 5HTP appears

to be specific since the fever producing pyrogens administered to LSD-tolerant animals showed hyperthermia comparable to that shown in control rabbits.

Discussion. These experiments demonstrate the pyretogenic property of 5HTP. It is presumed that this pyretogenic effect is due to the serotonin formed from the injected 5HTP. The fact that iproniazid, a potent brain monoamine oxidase inhibitor greatly potentiates the pyretogenic response supports this assumption since serotonin is deaminated by this enzyme. Furthermore the recent findings of Udenfriend *et al.* (1957a) indicate that serotonin formation proceeds at a rapid rate in the brain and other tissues after the administration of 5HTP. The rate of serotonin formation as found by these authors corresponds well with the onset of the pyretogenic effect.

The studies of the BOL antagonism indicate that the mechanism of the pyretogenic actions of LSD and 5HTP may be similar in nature. We have found that BOL does not affect pyrogen hyperthermia; it appears thus to have a considerable specificity against the actions of serotonin and other related compounds. The recent studies of Welsh and McCoy (1957) on the antagonism of LSD and serotonin by BOL in the *Venus mercenaria* heart also supports this. The antagonism which is exerted by BOL against both LSD and serotonin appears to be one of competitive nature since the administration of larger doses of the pyretogenic agent overrides the block and produces a temperature rise.

Frequently drugs of related structure demonstrate a cross tolerance after prolonged or repeated administrations. This is seen with the barbiturates or with the morphine-like analgesics as well as with certain sympathomimetic amines. In all of these cases the cross tolerance seems to be quite specific for certain structural characteristics of the drug molecule. The cross tolerance

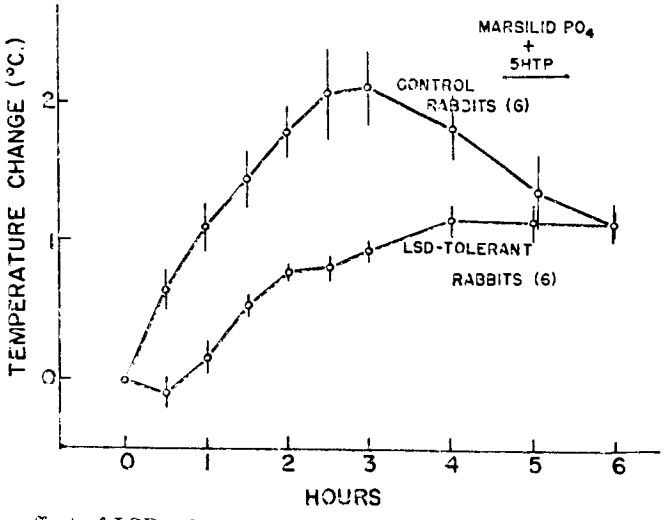

Fig. 4. The effect of LSD-tolerance on the pyretogenic effect of 5-hydroxytryptophan. Iproniazid, 40 mgm. per kgm., was given 1½ hours prior to the injections of 25 mgm. per kgm. of 5HTP to control and LSD-tolerant rabbits. Each curve represents the mean temperature change of six rabbits. Vertical lines indicate the standard error of the mean temperature change. Figures in brackets indicate the number of animals used in each group.

observed between LSD and 5HTP is probably of the same sort. It is a specific phenomenon since administration of pyrogens to LSD-tolerant animals produced a temperature rise of normal intensity.

The peripheral anti-serotonin actions of LSD earlier led to the hypothesis that its central nervous system effects might also be attributable to such a mechanism (Woolley and Shaw, 1954). Later studies by Cerletti and Rothlin (1955) indicated that this effect of LSD could not be responsible for the psychic actions in man. They found that BOL, an even more potent serotonin antagonist, did not produce abnormal psychic effects in man, even in large doses. On the other hand Ginzel and Mayer-Gross (1956) found that by pretreating subjects with BOL for several days, it was possible to antagonize the LSD symptoms in man. The additional evidence by Welsh and McCoy (1957) as well as the present studies in rabbits indicate that rather than acting as a serotonin antagonist, LSD produces symptoms resembling those of high brain levels of serotonin. While serotonin in lower concentrations produces a sedative action, in high doses a marked excitation is seen. LSD, on the other hand, in the rabbit appears to produce excitatory effects at all doses. In several other species, however, smaller doses of LSD show definite signs of sedation. Only upon increasing the dose is it possible to produce the excitatory response.

SUMMARY

5-Hydroxytryptophan administered to rabbits produces a pyretogenic response which is roughly proportional to the dose administered. Iproniazid potentiates this response markedly, indicating that serotonin is the agent directly responsible for the effect.

Both the LSD and 5HTP pyretogenic effects are greatly attenuated by prior administration of BOL, the bromine analogue of LSD. This block appears to be quite specific and is competitive in nature.

After tolerance is developed in rabbits by daily injections of progressively increasing doses of LSD the usual 5HTP pyretogenic response is greatly diminished, presumably due to a cross tolerance phenomenon.

These experiments suggest that the LSD pyretogenic effect is similar to that produced by high brain levels of serotonin.

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