BROM-LYSERGIC ACID DIETHYLAMIDE: A HIGHLY POTENT SEROTONIN ANTAGONIST

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Evidence that serotonin participates in normal physiology is accumulating, but its role or roles are not yet defined. Most recently, serotonin has been suggested as playing a part in central nervous system function. This hypothesis developed from the observations: 1) that LSD (d-lysergic acid diethylamide) an ergotamine derivative, blocks the central nervous system depressant action of serotonin in rats (Shore, Silver and Brodie, 1955); 2) that serotonin is found in the brain (Amin, Crawford and Gaddum, 1953; Twarog and Page, 1953); 3) that LSD induces a schizophrenic-like state (Stoll and Hofmann, 1943); 4) that serotonin may be concerned in nervous transmission in some invertebrates (Welsh, 1953; Florey and Florey, 1954); 5) that LSD blocks the action of serotonin on smooth muscle (Gaddum, 1953).

Gaddum and Hameed (1954) have assayed the serotonin blocking action of a series of ergotamine derivatives and found LSD to be the most active of the group. A new derivative with a bromine group in the 2 position, (2-brom-d-lysergic-acid diethylamide) BOL 148, has been synthesized.[2]

In the studies reported here this change in molecular structure is shown to enhance the compound's serotonin-blocking action. BOL causes complete blockade of serotonin in guinea pig ileum and uterus where LSD is without effect.

METHODS. The serotonin-blocking action of BOL was measured in three different smooth muscle preparations in a 10 ml. bath through which oxygen bubbled continuously.

A) *Rat uterus.* Female rats (200–250 gm.) were used 24 hours after subcutaneous injection of stilbestrol (10 microgm./100 gm. body weight) (Gaddum, Peart and Vogt, 1949). All animals were killed by concussion. A 2–3 cm. longitudinal strip of uterus was anchored at one end to a stainless-steel wire in a bath kept at 29–30°C. containing de Jalon, Bayo and de Jalon's solution (1945) (1 liter contains NaCl 9 gm., KCl 0.42 gm., $CaCl_2$ 0.06 gm., $NaHCO_3$ 0.5 and glucose 0.5 gm.). The other end of the strip was attached to a balanced frontal writing lever giving approximately five times magnification; responses were recorded on a smoked drum.

Strips that did not respond to at least 1–2 nonograms/ml. (0.001–0.002 microgm.) of serotonin, or which gave spontaneous contractions were discarded. Others strips showed good responses for as long as six hours.

B) *Guinea pig ileum.* The terminal part of the ileum with approximately 0.5 cm. openings made in the two sides was used. The bath was filled with a Tyrode solution containing NaCl

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8 gm., KCl 0.2 gm., $CaCl_2$ (anhydrous) 0.2 gm., $MgCl_2$ 0.1 gm., $NaHCO_3$ 1.0 gm., NaH_2PO_4 0.05 gm. and glucose 1.0 gm. per liter. Bath temperature was 36°C.

C) *Rabbit intestine.* Strips of duodenum 2–3 cm. long were suspended in Tyrode's solution of the same composition as that employed for guinea pig's ileum. The temperature of the bath was 36°C.

In all experiments, drugs were added to the bath every 5 minutes in volumes of from 0.05 to 0.2 ml. Serotonin, epinephrine and norepinephrine were left in contact with the preparation for 2 minutes; histamine and acetylcholine for 1 minute. The muscle strip was then washed four or five times. Serotonin concentration is given in terms of serotonin creatinine sulphate.

RESULTS. A) *Rat uterus.* Serotonin was added to the bath in an amount yielding a final concentration of one to two nonograms/ml.; these produced large but sub-maximal contractions. Tachyphylaxis was not observed when serotonin was injected every 5 minutes.

The blocking action of BOL was measured only after at least two consecutive responses of equal magnitude to serotonin. In a concentration of one to two nonograms/ml. it reduced contractions produced by serotonin. After thorough washing, the blocking action was more prominent than when BOL was in contact with the strip. The same is true for LSD. Complete blockade of serotonin-response occurred consistently when the concentration of BOL in the bath was approximately ten times that of serotonin; these concentrations of BOL did not themselves affect the strip but much larger doses (0.5–1.0 microgm./ml.) produced spontaneous contractions.

LSD was not as effective in blocking the action of serotonin as was BOL. Added to the bath in the same concentrations (10–20 nonograms/ml.), LSD diminished but did not block completely the effect of from 1 to 2 nonograms/ml. of serotonin, while BOL consistently caused a complete blockade. Further, these concentrations of LSD provoked spontaneous contractions of the muscle strip making interpretation of results difficult.

The blocking action of BOL was specific for serotonin; pitressin and acetylcholine continued to elicit normal contractions when response to serotonin was

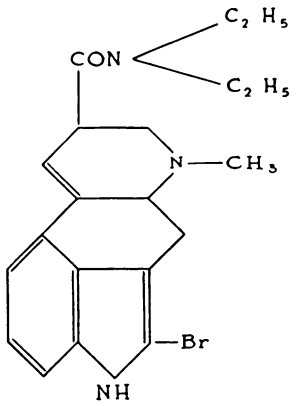

FIG. 1

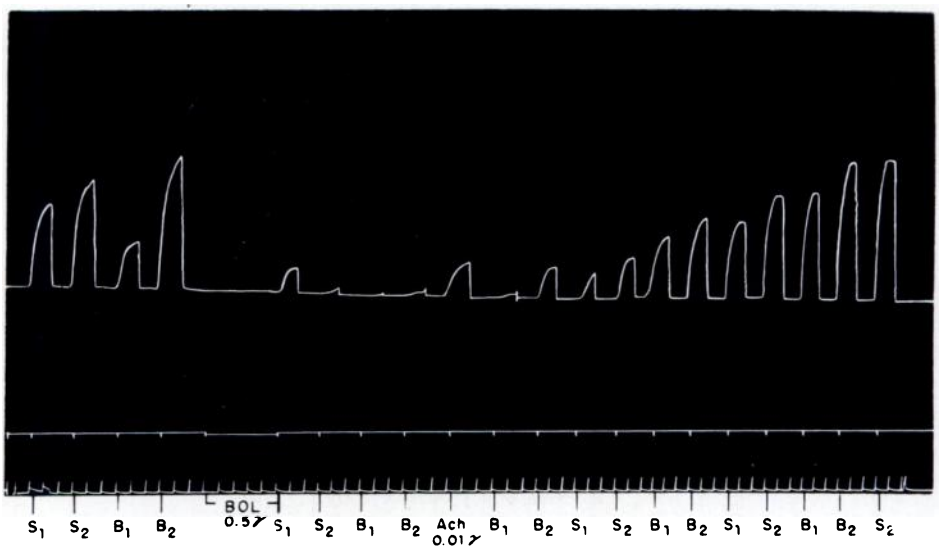

FIG. 2. Rat's uterus. Blockade of contraction due to serotonin and bufotenine by BOL. S_1-serotonin, 10 nonograms; S_2-serotonin, 20 nonograms; B_1-bufotenine, 0.5 microgm.; B_2-bufotenine, 1 microgm.; BOL, 0.5 microgm. Shows gradual recovery, complete 2 hours after BOL. Bath's capacity, 10 ml. Time marks, 1 minute.

absent. Marked increase in serotonin dosage would cause contraction of the muscle strip, indicating that the blocking action of BOL is probably a competitive one. It is temporary and recovery time was directly correlated with dosage. For example, with BOL concentrations of from 1 to 2 nonograms/ml., and partial serotonin blockade, recovery time was ten to fifteen minutes; with concentrations of from 10 to 20 nonograms/ml. recovery time was sixty to ninety minutes (figure 2).

BOL also prevented the contractions of the uterine strip produced by injection of bufotenine, a compound differing from serotonin in having two additional methyl groups. Bufotenine is about one hundred times less active than serotonin on the rat uterus, necessitating doses of from 100 to 200 nonograms/ml. to give the same contractions produced by 1 to 2 nonograms/ml. of serotonin. BOL was more effective against bufotenine than serotonin: 5 nonograms/ml. partially blocked the action of 1 to 2 nonograms of serotinin but blocked completely the action of 1 to 200 nonograms/ml. of bufotenine.

B) *Guinea pig ileum and uterus.* Serotonin (1 to 2 microgm./ml.) produced contractions in ileum approximately similar to those produced by histamine, acetylcholine or nicotine. Tachyphylaxis to serotonin was not observed when the drug was added to the bath every 5 minutes. The preparation was not used unless contractions were at least 2 to 3 cm. and unless spontaneous contractions were absent. Since most evidence indicates that serotonin acts upon receptors different from those stimulated by histamine or acetylcholine, one of the latter drugs was used in each experiment to ascertain that the blocking action of BOL was specific

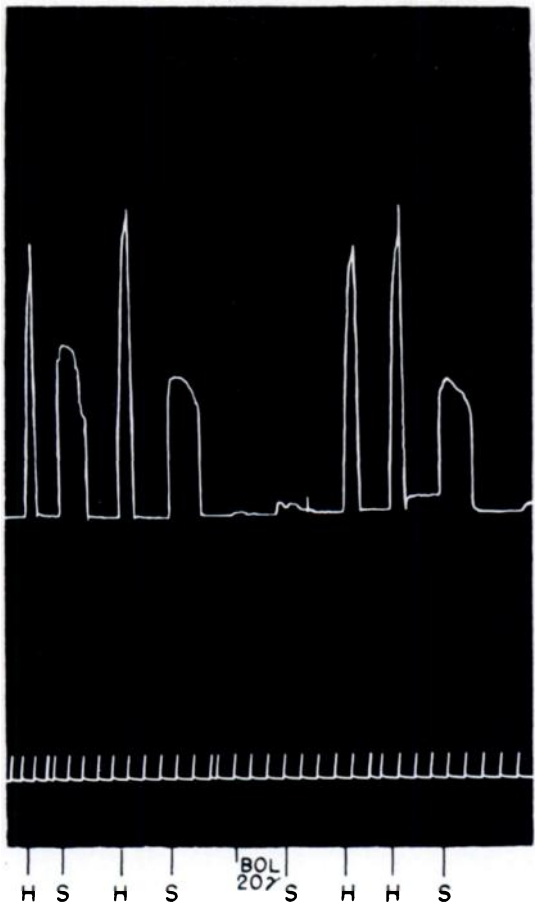

FIG. 3. Guinea pig's ileum. Blockade by BOL of contraction due to serotonin. H-histamine, 2 microgm.; S-serotonin, 10 microgm. BOL, 20 microgm. in contact with strip for 3 minutes. Note lack of effect on response to histamine. Recovery to serotonin returned 20 minutes after injection of BOL. Bath's capacity, 10 ml. Time marks, 1 minute.

for serotonin. We have been able to confirm Gaddum's observation that LSD does not block serotonin. With larger concentrations (10 to 20 microgm. ml.) of LSD, spontaneous contractions occurred.

As shown in figure 3, addition of 2 microgm./ml. of BOL prevented completely the response to 1 microgm. ml. of serotonin without affecting that to histamine. With this dosage of BOL, recovery time was about fifteen minutes. In doses of 1 to 2 microgm. ml., BOL had no effect on the preparation; in larger doses (5 to 10 microgm. ml.) it caused spontaneous contractions which persisted even after repeated washings. The response of the guinea pig uterus to serotonin was also blocked by BOL.

C) *Rabbit's intestine.* Serotonin caused increase of tonus and pendular movements fifteen to thirty seconds after being added to the bath, and these effects

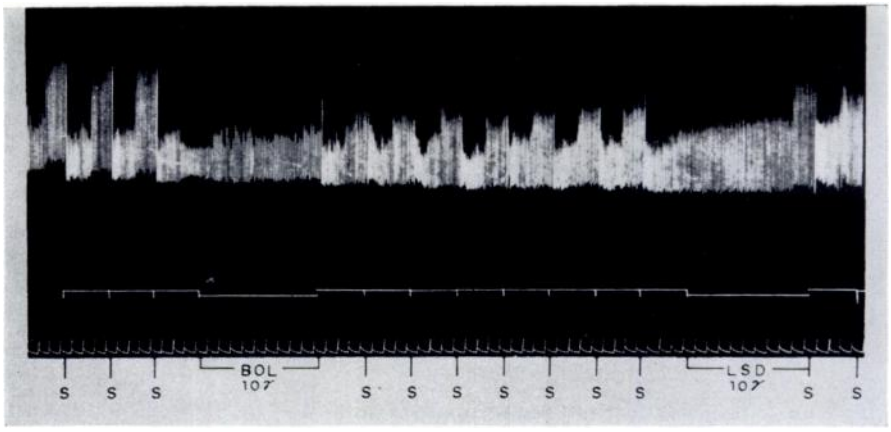

FIG. 4. Rabbit's duodenum. Response to 5 microgm. of 5-serotonin, and its blockade by BOL, 10 microgm. Recovery in 1 hour. LSD, 10 microgm. then injected, did not block the response to serotonin. Bath's capacity, 10 ml. Time marks, 1 minute.

remained for two to five minutes. This response was reproducible provided that there was an interval of 5 minutes between injections.

BOL (concentrations of 1 to 2 microgm./ml.) reduced the peristaltic movements not only in intensity but also in duration, and such doses prevented the effects of serotonin without changing those of acetylcholine, histamine, epinephrine or norepinephrine.

Figure 4 shows a representative experiment in which BOL (1 microgm./ml.) blocked the contraction produced by serotonin (1 microgm./ml.) with recovery in 15–20 minutes. In the same experiment LSD (1 microgm./ml.) had no effect on response to serotonin.

DISCUSSION. The introduction of a bromine atom into the lysergic acid diethylamide molecule is shown to enhance markedly its ability to antagonize the action of serotonin. Using the rat's uterus as an indicator, BOL is the most potent known inhibitor of serotonin. Concentrations of only 1 to 2 nonograms/ml. reduced contractions due to serotonin and complete blockade occurred when the concentration of BOL was approximately ten times that of serotonin.

The difference in action between LSD and BOL is even more striking when the guinea pig ileum is used as an indicator. Gaddum and Hameed (1954) found that LSD was an active and specific antagonist to the action of serotonin on the rat's uterus and rabbit's ear vessels but that it had little effect on guinea pig's ileum, even when high concentrations were used. We have confirmed the latter observation. They suggested that there were two types of tryptamine receptors: (a) those in smooth muscle of rat's uterus and rabbit's ear vessels which are easily paralysed by LSD but not so easily paralysed by excess of serotonin; (b) receptors in the ganglia of the intestine which are easily paralysed by an excess of serotonin but not by LSD. Since we find that BOL blocks the action of serotonin in guinea pig ileum even though LSD is ineffective on this preparation,

there now seems no need to postulate more than one type of receptor. We suggest the following possibilities:

(a) Receptors of guinea pig intestine are less sensitive to serotonin than those in the rat's uterus, requiring a higher density of serotonin molecules to elicit contraction. LSD being a less effective competitor than BOL would require higher concentrations to be effective. But high concentrations of LSD of themselves cause contractions of the intestine. Thus, this hypothesis cannot be tested experimentally.

(b) The presence of bromine in the BOL molecule may enable this compound to penetrate receptors more effectively.

SUMMARY AND CONCLUSIONS

1. 2-Brom-d-lysergic acid diethylamide (BOL) is a more potent serotonin antagonist than lysergic acid diethylamide (LSD) on the rat's and guinea pig's uterus as well as on guinea pig's ileum and rabbit's intestine.

2. In these experiments, as in those of Gaddum and Hameed, LSD proved an active and relatively specific antagonist to serotonin on estrous rat's uterus but failed to oppose the action of serotonin on guinea pig's ileum. It also failed in these experiments to block the action of serotonin on rabbit's intestine. BOL, on the other hand, effectively blocked the action of serotonin on both guinea pig's ileum and rabbit's intestine and its action is relatively specific in that it does not reduce the effect of acetylcholine, histamine, epinephrine or norepinephrine.

3. There seems no need to postulate two sets of serotonin receptors to explain the failure of LSD to block the action of serotonin on guinea pig ileum. The greater effectiveness of BOL as a blocking agent suffices to explain its ability to oppose the action of serotonin on guinea pig ileum and rabbit's intestine.

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