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PRELIMINARY NOTE

RELEASE OF 5-HYDROXYTRYPTAMINE FROM SLICES OF SUPERIOR COLLICULUS BY OPTIC TRACT STIMULATION

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Summary—Slices of guinea pig superior colliculus were incubated in an artificial medium with labelled 5-hydroxytryptamine (^{3}H-5-HT). After incubation, electrical stimulation was applied to the optic tract and efflux of ^{3}H-5-HT was measured in the superfused medium. During the stimulation period a marked increase of ^{3}H-5-HT was observed. This increase of ^{3}H-5-HT was not found when LSD was applied in the incubation medium or when the optic tract was cut.

EVIDENCE has been accumulated that 5-hydroxytryptamine (5-HT, serotonin) is a transmitter in the central nervous system. The presence of 5-HT-containing nerve terminals has been demonstrated in the mammalian brain (MICHAELSON and WHITTAKER, 1962). By electrical stimulation of nervous tissue, depletion of serotonin (DAHLSTRÖM *et al.*, 1965) and release of serotonin from brain slices (CHASE *et al.*, 1968, 1969; KATZ and KOPIN, 1969) has been demonstrated. But the data indicating release of 5-HT after axonal stimulation are still lacking.

It has already been reported that the postsynaptic potential evoked in a slice of the superior colliculus in response to optic tract stimulation was suppressed by an application of 5-HT, and this suppression was blocked by LSD (KAWAI and YAMAMOTO, 1969). Since catecholamine had no effect on this potential it can be considered that 5-HT has a specific action on this structure.

The present experiments have been performed to confirm the release of 5-HT from the superior colliculus in response to optic tract stimulation and to study the effect of LSD on the release of 5-HT.

Slices of guinea pig superior colliculus including the incoming optic tract were prepared as described by YAMAMOTO and MCILWAIN (1966). The slice was incubated at 37°C in a bath with 2ml of glucose–saline medium (YAMAMOTO and KAWAI, 1967) saturated with 95% O_2 and 5% CO_2, containing tritiated 5-HT (5·6 Ci/mM, 1 mμg and 10 mμ Ci/ml) (The Radiochemical Centre, Amersham, England). After incubation for 30 min, the slice was flushed with fresh medium. To rinse the tritium from its surface, the slice was soaked in fresh medium. Every 10 min, the medium bathing the slice was replaced with fresh medium and

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serial fractions were collected. Radioactivity of each fraction was assayed by liquid scintillation spectrometry (Tri Carb, Packard, U.S.A.). ^{3}H-5-HT in the medium was separated from its metabolite, ^{5}H-5-hydroxyindoleacetic acid (^{3}H-5-HIAA), by adsorption on cellulose phosphate and selective elution with ammonium acetate in concentration of 0·1 M (5-HIAA) and 1 M (5-HT), respectively (BLACKBURN *et al.*, 1967). Square pulses of 0·1 msec in duration and 10–20 V in strength were used to stimulate the optic tract. A recording electrode was placed on the superior colliculus to monitor the evoked potentials.

Figure 1A shows the results of a typical experiment. The spontaneous efflux rate of tritiated 5-HT was high immediately after incubation with ^{3}H-5-HT (Block 1) and it gradually decreased as the time elapsed. When electrical stimulation was applied to the optic tract

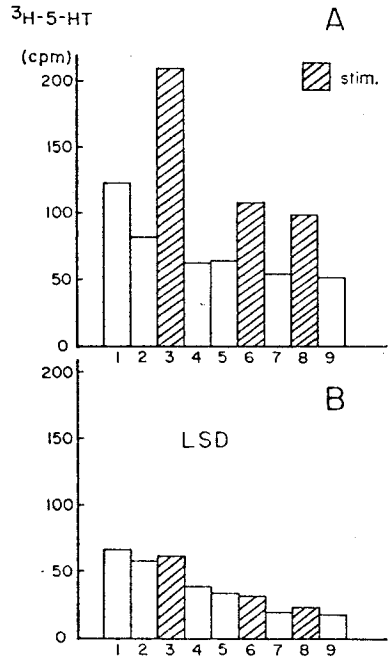

FIG. 1. A. Release of tritiated 5-HT from a slice of the superior colliculus. Each block shows the amount of ^{3}H-5-HT (counts/min/2ml medium) released in 10 min period. Blocks with oblique lines denote the efflux of ^{3}H-5-HT during stimulation applied to the optic tract at a rate of 5 c/s.

B. Result from another slice showing the effect of LSD on the release of 5-HT. LSD (1×10^{-6}M) was applied to the incubation medium 3 min before addition of ^{3}H-5-HT.

(5 c/s, 10 min), the efflux rate of ^{3}H-5-HT sharply increased (Block 3). During successive periods of stimulation, the amount of ^{3}H-5-HT released by electrical stimuli was also increased (Block 6,8) but by a lesser extent than that observed after the first stimulation (Block 1).

In order to test whether 5-HT was released only from the optic tract axons terminals in the superior colliculus, after incubating the brain slice in ^{3}H-5-HT we severed the optic tract near its entrance in the superior colliculus. After this procedure, no increase in ^{3}H-5-HT efflux was elicited by the electrical stimulation of the optic tract. In this preparation it was found that the presynaptic action potentials were unaltered by the optic nerve transection.

In fact, we could monitor the axonal potentials with an electrode placed on the optic tract proximal to the cut end. These results showed that the increase of tritium efflux shown in Fig. 1A (Block 3,6 and 8) was not an artefact caused by the electric current.

LSD in concentrations around 1×10^{-6} M blocks the 5-HT action in the superior colliculus (KAWAI and YAMAMOTO, 1969). Figure 1B shows an experiment in which LSD in a concentration of 1×10^{-6} M was given prior to the addition of tritiated 5-HT to the incubation medium. As illustrated in Block 1, 2 of Fig. 1B, spontaneous release of ^{3}H-5-HT was noticeably reduced compared to that in Fig. 1A and the stimulation of the optic tract brought about no definite increase in ^{3}H-5-HT (Block 3,6,8 in Fig. 1B). Bufotenine, a psychotomimetic agent, had been found to block the 5-HT action as LSD did (KAWAI and YAMAMOTO, 1969). In the present experiment, after bufotenine (1×10^{-5} M) was added to the incubation medium, the spontaneous as well as the electrically-induced ^{3}H-5-HT efflux was reduced just as in the case of LSD.

When the concentration of Ca^{2+} in the medium was lowered to 1/10 (0·26 mM) of normal, postsynaptic potentials recorded from the superior colliculus were markedly reduced, while the presynaptic action potentials remained unchanged. In this low-Ca^{2+} medium, spontaneous release of 5-HT was reduced to some extent but the amount of electrically-induced release of ^{3}H-5-HT was much the same as that in normal medium.

The present experiments gave evidence that 5-HT was released from the superior colliculus in response to optic tract stimulation. Since no increase of ^{3}H-5-HT was obtained in the slice in which the superior colliculus had been isolated from the optic tract, two sites can be considered to be involved in the release of 5-HT by optic tract stimulation; presynaptic terminals or postsynaptic membranes of the neurons in the superior colliculus. In view of the findings reported by several authors that the labelled 5-HT was largely taken up in the nerve terminals (AGHAJANIAN and BLOOM, 1967; BLACKBURN *et al.*, 1967), the presynaptic nerve terminals are probably the site responsible for release of 5-HT.

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