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RELEASE OF NOREPINEPHRINE-^{3}H AND SEROTONIN-^{3}H EVOKED FROM BRAIN SLICES BY ELECTRICAL-FIELD STIMULATION—CALCIUM DEPENDENCY AND THE EFFECTS OF LITHIUM, OUABAIN AND TETRODOTOXIN

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Abstract—Slices of mammalian brain accumulate tritiated norepinephrine or serotonin when incubated in a medium with these compounds. Mild electrical stimulation of short duration induces a striking increase in the release of exogenous-labeled amine. Electrically stimulated release of norepinephrine-^{3}H, but not of serotonin-^{3}H, is calcium dependent. Stimulation-induced release of both monoamines is significantly diminished by the addition of ouabain, lithium or tetrodotoxin to the perfusing medium. Elevated calcium concentration prevents lithium-induced inhibition of norepinephrine-^{3}H release but has no effect on lithium inhibition of evoked release of serotonin-^{3}H. Enhanced calcium levels did not reverse ouabain-induced inhibition of release of either monoamine.

THERE is considerable evidence to support the view that both norepinephrine[1, 2] and serotonin[3–5] are central neurotransmitters. Studies *in vitro* have demonstrated that slices of brain actively accumulate both monoamines[6, 7] in nerve endings. Electrical stimuli of low intensity and short duration, which are known to depolarize nerve membranes,[8, 9] markedly enhance release of norepinephrine-^{3}H and serotonin-^{3}H from brain slices into a perfusing medium.[10, 11] The comparative ionic requirements and the effects of drugs on this release have been examined.

METHODS

Adult male Sprague–Dawley rats weighing 150–200 g were killed by cervical fracture and their brains rapidly removed. Slices of tissue were prepared from the brain as follows: a coronal section of brain was made about 4 mm from the anterior pole of the cerebral hemispheres and a coronal slice about 0·5 mm thick was cut off from the posterior portion. The slice was placed on a piece of wet (Krebs–Ringer bicarbonate solution) filter paper, and a disc of tissue 3 mm in diameter (weighing about 20 mg) was punched out of the area corresponding to the striatum. Such discs were placed in 20 ml of a solution containing the following ions (m-equiv./l): sodium (141), potassium (5·93), calcium (5·08), magnesium (2·36), chloride (104·8), phosphate (2·22), sulfate (2·36) and bicarbonate (2·49); pyruvate (4·9), glutamate (4·9), fumarate (5·4) and glucose (9·2) were used as energy sources.[12] The solution was saturated with

5% carbon dioxide in oxygen and maintained at 37°. Serotonin-^{3}H (5HT-H3) (2 c/mm, 50 ng/ml) and norepinephrine-^{3}H (NE-H3) (8 c/mm, 25 ng/ml) were added and incubation was continued for 30 min, after which the tissues were transferred to glass-superfusion chambers.

The glass-superfusion chambers consisted of open-end tubes (0·5 cm diameter, 2·5 cm length) mounted vertically by sealing the open ends into holes of the upper and lower plates of a rectangular, clear-lucite box. The lucite box served as a jacket through which water at 37° was pumped. The open ends of the superfusion chambers were sealed with small rubber plungers, each of which held a small metal tube connected to a polyethylene tube through which the superfusing solution could be introduced (at the bottom) and collected (from the top). Platinum wires (No. 22) which ended in flat coils (about 0·4 mm diameter) served as electrodes and were adjusted so that the brain slice rested between the electrodes, which were placed about 1·5 mm apart.

Fresh, warmed, oxygenated medium was pumped through the chambers at a rate of about 0·3 ml/min so that the fluid surrounding the tissue was rapidly replaced (volume of chamber was less than 0·4 ml). When spontaneous efflux of radioactivity into the effluent fluid diminished to low, relatively constant levels (20 min), rectangular DC pulsatile stimulation (10 mA, 100 cycles/sec, 4·0 m-sec duration) from a Grass S-4 stimulator through a constant current control unit was applied through the platinum electrodes for a total of 1 min.

Effluent superfusate was collected during 2-min intervals immediately before and during stimulation and assayed for total tritium by liquid scintillation spectroscopy.

In some experiments, stimulus-evoked release of the tritiated monoamines was studied with various changes in the ionic composition or after drugs were added to the superfusing medium. In these studies, tissues were incubated in control medium and exposed to the altered solution throughout the perfusion, stimulation and collection periods. The following drugs were used: ouabain, lithium chloride or tetrodotoxin. In the experiments in which "low calcium solution" was used, 0·1% sodium ethylenediaminetetra acetic acid (NaEDTA) was present in calcium-free Krebs–Ringer bicarbonate superfusing medium.

RESULTS

Effect of omitting calcium on release of amines. Omission of calcium ions from the perfusing medium had no effect on the rate of spontaneous efflux of tritiated amines from brain slices, nor did low calcium concentration appear to alter stimulation-induced release of serotonin-^{3}H (Fig. 1). However, in the absence of calcium, evoked release of norepinephrine-^{3}H was markedly diminished.

Effects of ouabain and lithium with normal and high (11 mM) calcium concentrations on release of amines. When ouabain (10^{-4} M) or lithium chloride (2·4 m-equiv./l) was present in the superfusate, there was no alteration in the rate of spontaneous efflux of the labeled amines in the interval just before stimulation. Stimulation-induced amine release, however, was significantly diminished by each agent (Fig. 2).

Increasing the concentration of calcium (11 mM) in the superfusate did not alter amine release in the absence of drugs nor in combination with ouabain. Augmented levels of calcium, however, did prevent the lithium-induced diminution of release of

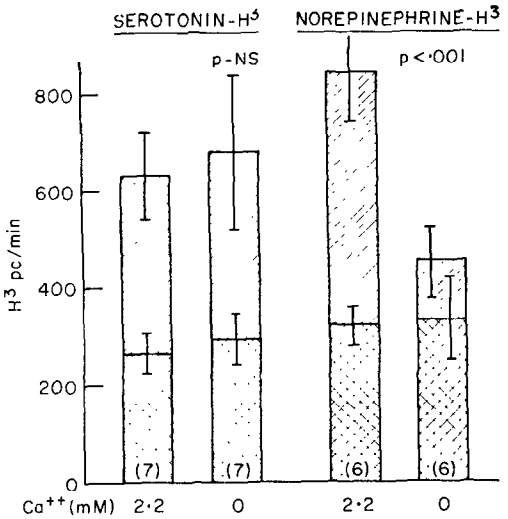

FIG. 1. Effect of low calcium on release of amines. Slices of striatum were incubated with labeled amines, superfused and stimulated as indicated in the text. The cross-hatched area of the bars indicates the levels of tritium efflux before stimulation; the total height, release during stimulation; the lined areas, therefore, represent stimulation-induced release. Results are expressed as picocuries per min and are the means ± S.E.M. for the number of slices shown in parentheses at the foot of each bar. The P values refer to differences in stimulation-induced release of tritium. Concentration of calcium ion in the perfusing solution is indicated on the abscissa.

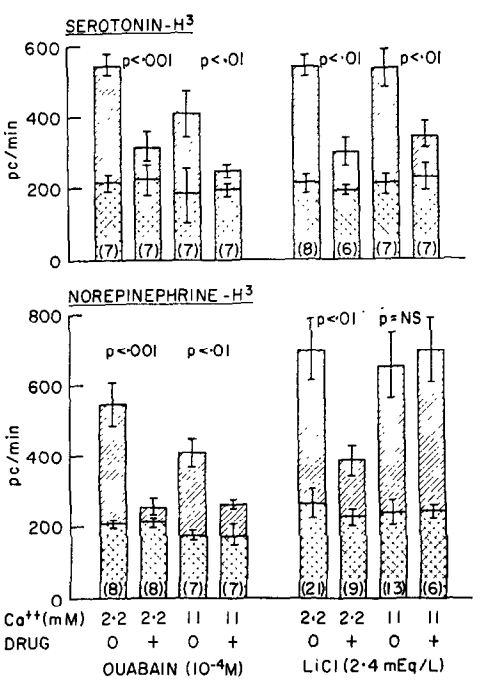

FIG. 2. Effect of drugs and calcium on release of amines. Slices of striatum were incubated with labeled amines, superfused and stimulated as indicated in the text. Results are expressed as described in Fig. 1. Drug and ion concentrations are indicated on the abscissa.

norepinephrine-^{3}H but failed to reverse the inhibition of stimulation-induced release of serotonin-^{3}H by lithium.

Effect of tetrodotoxin on stimulus-evoked release of monoamines. The presence of tetrodotoxin (10^{-6} g/ml) in the superfusing medium significantly diminished evoked release of both monoamines at a stimulus strength of 10 mA (Fig. 3). When the current was increased to 25 mA, no inhibitory effect was observed.

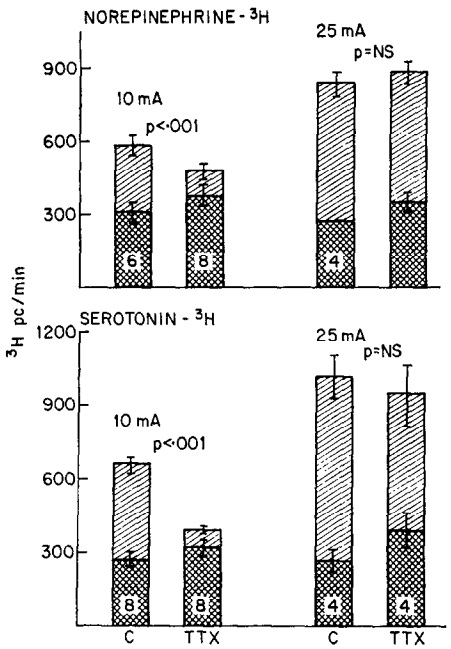

FIG. 3. Effect of tetrodotoxin on evoked release of ^{3}H-monoamines from rat striatal slices. Slices of striatum were incubated with labeled amines, superfused and stimulated as indicated in the text. Results are expressed as described in Fig. 1. The concentration of tetrodotoxin was 10^{-6} g/ml.

DISCUSSION

Slices of brain actively accumulate norepinephrine-^{3}H from incubation media[6] and localize it in granules in nerve endings.[13] Similarly, serotonin-^{3}H is accumulated by brain slices[7] and appears to be concentrated largely within nerve terminals which normally contain this indoleamine.[14, 15] The remarkably good agreement between the effects of drugs on uptake of biogenic amines *in vitro* and their effects *in vivo*[16] suggests that the depolarization-induced release observed in these experiments may closely simulate the nerve impulse-induced release occurring in the intact brain.

Lithium salts appear to be effective in the treatment of mania and hypomania[17, 18] and to influence the metabolism of norepinephrine *in vivo*.[19] Lithium at a concentration of 2·4 m-equiv./l., comparable to that found in the brain 8 hr after injection of 7·5 m-equiv./kg,[20] partially inhibits electrically induced release of both NE-H3 and 5HT-H3 from brain slices (Fig. 2).

The mechanism of release of norepinephrine from the adrenal medulla,[21] peripheral sympathetic nerve[22] and brain slice[10] (Fig. 1) is known to involve calcium. Since the

effect of lithium on NE-H3 was reversed by augmented calcium concentration (Fig. 2), it appears that lithium may act directly or indirectly to interfere with calcium participation in stimulus-release coupling. In contrast to the inhibitory effect of low calcium on the release of NE-H3 from brain slices, the absence of calcium in the superfusate does not appear to interfere with the release of 5HT-H3 (Fig. 1). Thus it appears that the mechanism of release of serotonin is different from that of norepinephrine. The observation that lithium inhibited evoked release of 5HT-H3 and was not reversed by increasing calcium levels is consistent with the observation that serotonin release is not calcium dependent (Fig. 1). The inhibition of the noncalcium-dependent release of serotonin suggests that lithium may not diminish norepinephrine release by a direct effect on calcium.

Ouabain (10^{-4} M) has been shown to block uptake into brain slices of both norepinephrine[6] and serotonin.[7] At similar concentrations, this drug significantly diminished release of both putative neurotransmitters while augmented levels of calcium in the superfusing medium failed to reverse the drug-induced inhibition of release of either amine (Fig. 2). Ouabain blocks sodium-dependent axonal conduction and could interfere with conduction of a nerve impulse, generated in the axon, to the nerve terminal. The observation that tetrodotoxin also interferes with low amperage (10 mA) induced release is consistent with this view. This inhibitory effect of tetrodotoxin could be reversed by higher electrical currents (25 mA) (Fig. 2) which may directly depolarize the nerve varicosities.

These findings suggest that electrical-field stimulation-induced release of labeled monoamines from brain slices may serve as a useful model for studying central release mechanisms as well as for detailed observations of the mode of action of drugs affecting the central nervous system.

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