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SHORT COMMUNICATION

Cyclic Adenosine 3′,5′-Monophosphate Formation in Brain Slices: Stimulation by Batrachotoxin, Ouabain, Veratridine, and Potassium Ions

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SUMMARY

The formation of cyclic adenosine 3′,5′-monophosphate-^{14}C from endogenous ATP-^{14}C is greatly stimulated on incubation of cerebral cortex slices with depolarizing agents such as batrachotoxin, veratridine, ouabain, and potassium ion. In slices of cerebral cortex, batrachotoxin is the most potent known stimulant (ED_{50} = 1 × 10^{-7} M) of cyclic 3′,5′-AMP-^{14}C formation. Stimulation of cyclic 3′,5′-AMP-^{14}C formation by depolarizing agents requires calcium ions and is inhibited by theophylline. Stimulation of cyclic 3′,5′-AMP-^{14}C formation by histamine, in contrast, does not require calcium ions and is not inhibited by theophylline. The results suggest a relationship among depolarization, transmitter release, and cyclic 3′,5′-AMP formation in brain.

The formation of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in slices of brain tissue is stimulated by incubation with such possible neutrotransmitters as (nor)-epinephrine, histamine, and serotonin (1) and by electrical stimulation of the slices (2). These observations, the synaptic localization of brain adenyl cyclase (3), and the high levels of adenyl cyclase and cyclic 3′,5′-nucleotide phosphodiesterase in the central nervous system (4, 5) all suggest a key role for cyclic AMP in the regulation of brain function.

A sensitive radiometric technique may now be used with incubated brain slices to assess the effect of potential neurotransmitter substances on the formation of cyclic AMP. The method consists of labeling endogenous stores of ATP by a preliminary incubation with adenine-^{14}C, followed by incubation with test substances and radiometric assay of cyclic AMP-^{14}C after isolation by thin-layer chromatography (6). As measured by this method, norepinephrine, histamine, and various histamine analogues greatly stimulate cyclic AMP-^{14}C formation (6, 7).

We now wish to report that a variety of chemical agents, known to cause membrane depolarization and release of transmitter (acetylcholine) in nerve-muscle preparations, cause a profound stimulation of the formation of cyclic AMP-^{14}C in incubated slices of cerebral cortex. For example, high concentrations of potassium ion are known to cause depolarization of nerve membranes and to facilitate spontaneous release of neurotransmitter substances from the presynaptic terminals. Such spontaneous release of transmitter substances usually requires calcium ions (8). In brain slices, high concentrations

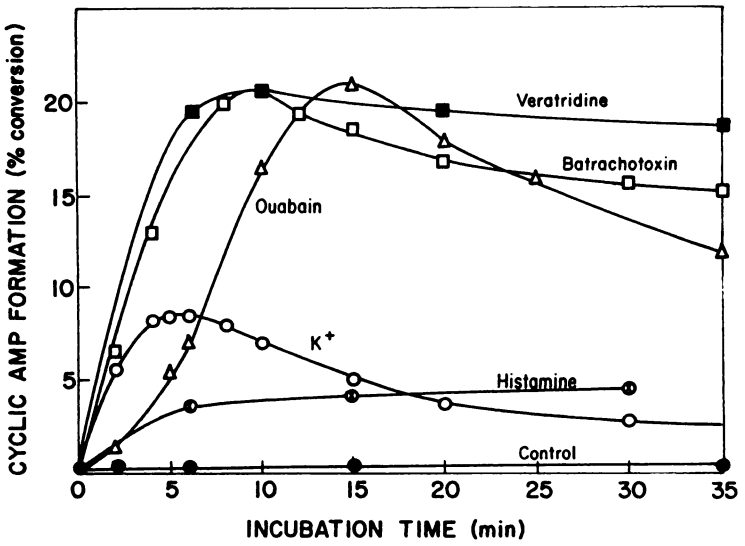

FIG. 1. *Time course for stimulation of cyclic AMP-^{14}C formation by depolarizing agents and histamine*
Approximately 1 g of cerebral slices of guinea pigs was pulse-labeled for 40 min with adenine-8-^{14}C (1.5 μCi, 0.133 μmole), divided into several portions, and incubated with a stimulant, and the formation of cyclic AMP-^{14}C was followed by radiometric assay, as previously described (6). Results shown are typical examples of the time course for cyclic AMP-^{14}C formation, expressed in terms of percentage of the total ^{14}C present in slices converted to cyclic AMP-^{14}C in the presence of ouabain (0.05 mM, △), batrachotoxin (0.002 mM, □), veratridine (0.05 mM, ■), high potassium ion levels (43 mM, ○), or histamine (1.0 mM, ⊗) and the standard control medium (NaCl, 122 mM; KCl, 3 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.3 mM; KH_2PO_4, 0.4 mM; D-glucose, 10 mM; $NaHCO_3$, 25 mM, ●).

of potassium ion cause enhanced release of newly synthesized acetylcholine, which is again dependent on the presence of calcium ions in the incubation medium (9, 10). A preliminary report indicated that endogenous levels of cyclic AMP were elevated in brain slices after incubation with high concentrations of potassium ions (11). The present results on cyclic AMP-^{14}C formation confirm this report and demonstrate that stimulated formation of cyclic AMP-^{14}C by high concentrations of potassium ion and other depolarizing agents requires the presence of calcium ions.

Three other depolarizing agents, batrachotoxin, ouabain, and veratridine, at low concentrations caused enhanced formation of cyclic AMP-^{14}C in incubated slices of cerebral cortex. The time course of formation of cyclic AMP-^{14}C in slices of guinea pig cerebral cortex stimulated by potassium ion, ouabain, veratridine, batrachotoxin, and, for comparison, histamine is presented in Fig. 1. With batrachotoxin, veratridine, and ouabain, a *maximum* of approximately 20% of the total radioactivity in slices can be converted to cyclic AMP-^{14}C in a period of 8–15 min. In subsequent experiments, formation of cyclic AMP-^{14}C was measured at a time corresponding to maximal stimulation with each of these agents. The total radioactivity remaining in the slice after incubation with depolarizing agents was only slightly less (approximately 10%) than in control slices.

Batrachotoxin is the *most potent known stimulant* of cyclic AMP formation in the cerebral cortex, with an ED_{50} of 1×10^{-7} M (Fig. 2). Veratridine and ouabain are much less potent. Slices of cerebral cortex from guinea pig are less sensitive to stimulation by histamine than those of rabbit, as seen in Figs. 2 and 3. Conversely, slices from rabbit cerebral cortex are less sensitive to depolarizing agents than slices from guinea pig (12).

Ouabain, in all likelihood, causes depolarization and release of acetylcholine in nerve-muscle preparations because of inhibition of sodium- and potassium-activated ATPase

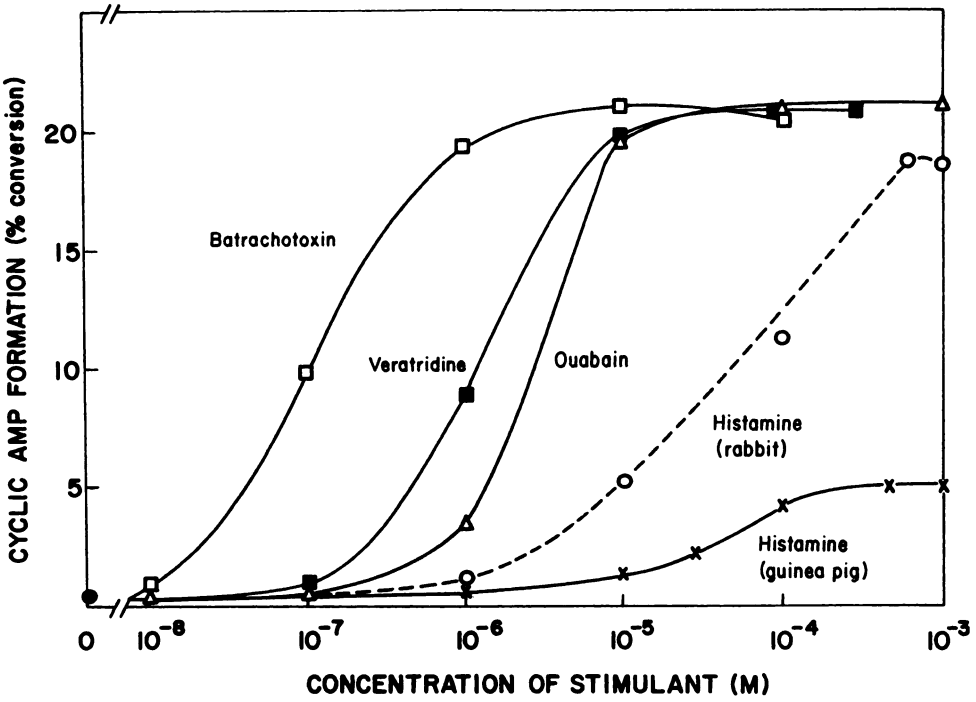

FIG. 2. *Dose-response curves for stimulation of cyclic AMP-^{14}C formation by depolarizing agents and histamine*

Cyclic AMP-^{14}C formation was assayed as described in Fig. 1. Cerebral slices were obtained from gray matter of guinea pigs and, for the histamine stimulation experiments guinea pigs and rabbits. Incubation time was 10 min for batrachotoxin (□), veratridine (■), and histamine (guinea pig, ×; rabbit, ○) and 15 min for ouabain (△).

and a resultant increase in levels of intracellular sodium ion (13). Veratridine, on the other hand, is thought to cause depolarization and transmitter release in nerve-muscle preparations because of its effect on the permeability of membrane to sodium ion (14). Batrachotoxin causes depolarization and transmitter release by selectively increasing the permeability of synaptic membranes to sodium ion (15). In spite of their differing modes of action, all three depolarizing agents cause a greatly enhanced formation of cyclic AMP-^{14}C in slices of cerebral cortex. In addition, they stimulate the release of newly synthesized acetylcholine from the slices (12).

Maximal stimulation of cyclic AMP-^{14}C formation is evoked by batrachotoxin at a concentration of 2×10^{-6} M. At this concentration, batrachotoxin only slightly inhibits the hydrolysis of cyclic AMP by phosphodiesterase from guinea pig brain, using the method of Cheung (16). Ouabain does not inhibit the phosphodiesterase at a concentration of 2×10^{-4} M. Inhibition of phosphodiesterase is therefore not involved in the stimulated formation of cyclic AMP-^{14}C by these depolarizing agents.

The enhanced formation of cyclic AMP-^{14}C elicited by batrachotoxin, ouabain, and veratridine is, as was the case for stimulation by potassium ion, dependent on the presence of calcium ions in the medium (Fig. 3). In contrast, histamine-evoked stimulation of cyclic AMP-^{14}C formation is not reduced after omission of calcium ions (Fig. 3). Since calcium ions are also necessary to link depolarization with transmitter release, it seems quite likely that the enhanced formation of cyclic AMP elicited by depolarizing agents is in some way associated with transmitter release. Whether enhanced formation of cyclic AMP is involved presynaptically in

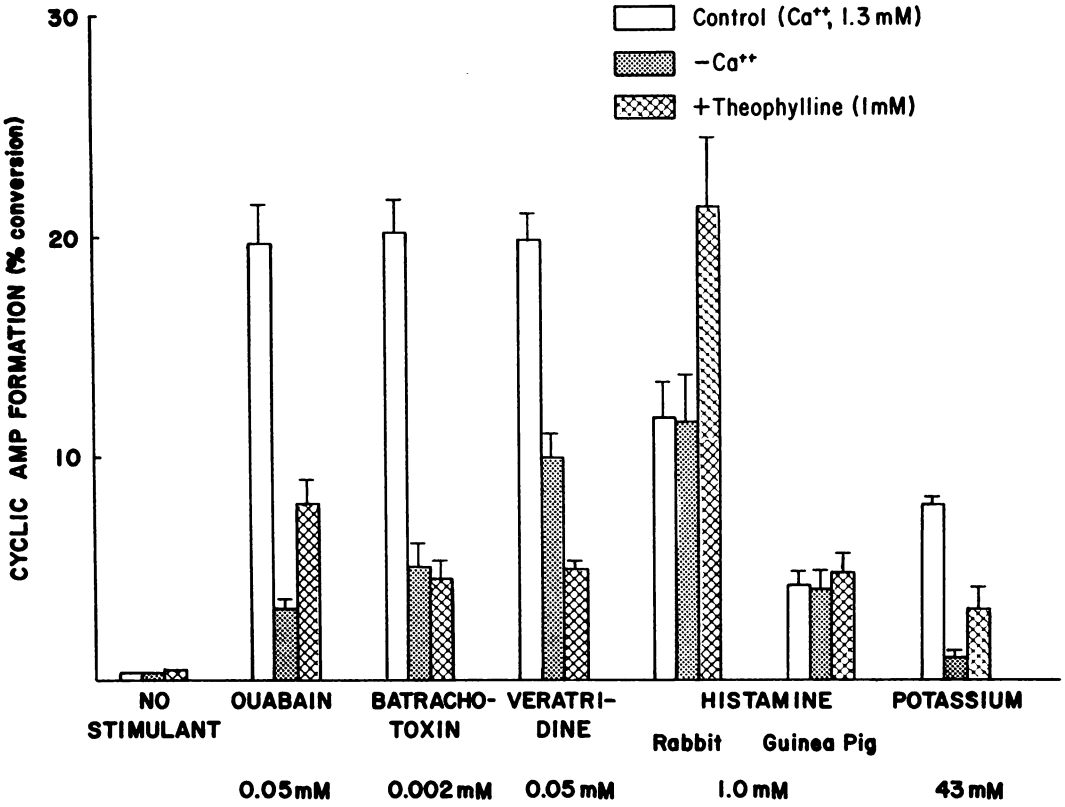

FIG. 3. *Stimulation of cyclic AMP-^{14}C formation by depolarizing agents and histamine; effects of calcium ions and theophylline*

The results are averages of more than three separate experiments ± standard deviation, shown by vertical lines. Incubation time was 7 min for potassium ion, 10 min for batrachotoxin, veratridine, histamine, and no stimulant, and 15 min for ouabain. Cerebral slices were obtained from guinea pigs and, for the histamine experiments, from both rabbit and guinea pigs. Calcium-free medium was prepared by omission of $CaCl_2$ from the control medium (Fig. 1). The effect of theophylline was studied at a final concentration of 1.0 mM.

the events leading to transmitter release (17, 18), or whether depolarization evokes the release of an unknown transmitter substance which then stimulates cyclic AMP formation postsynaptically, remains to be determined. Rall and Sattin (19) have proposed that electrical stimulation and high levels of potassium cause the release of adenosine or a related nucleotide, and that this compound then stimulates cyclic AMP formation.

Theophylline and caffeine prevent the further metabolism of cyclic AMP by phosphodiesterase. For this reason, theophylline often appears to potentiate the stimulation of cyclic AMP formation due to histamine (Fig. 3) and other stimulants. The stimulation elicited by depolarizing agents is, however, partially blocked by the presence of 1 mM theophylline (Fig. 3). The inhibitory effect of theophylline on cyclic AMP formation in brain slices has also been reported with electrical stimulation (2) and with adenosine (19). The adenosine-theophylline interaction has been confirmed using the present assay procedure (12).

Depolarizing agents such as potassium ion, ouabain, batrachotoxin, and veratridine, at their maximal concentrations, did not show an additive effect when combinations of any two of these agents were tested. However, combination of histamine (5×10^{-4} M) with any of the depolarizing agents caused a

35–40% conversion of total radioactivity to cyclic AMP-^{14}C in the cerebral slices of guinea pigs. This conversion is much greater than would be predicted from a simple summation of the separate effects of histamine and the depolarizing agent. Similar synergisms between the effects of either histamine or norepinephrine and electrical stimulation (2) and of norepinephrine and adenosine (19) on cyclic AMP formation have been reported. The extent of conversion of radioactive adenine nucleotides to cyclic AMP-^{14}C with various agents suggests that incubation of brain slices with adenine-^{14}C results in the labeling of a small, compartmentalized pool of adenine nucleotides, which are excellent precursors of cyclic AMP. This pool represents less than 10% of the total ATP of the slice and does not readily equilibrate with the remainder of the ATP stores (12).

Depolarizing agents can now be added to a growing list of compounds which stimulate formation of cyclic AMP in brain. In this regard, it is of interest that one of these agents, ouabain, *inhibits* formation of cyclic AMP in adipose tissue (20). The results with brain slices indicate that various depolarizing agents such as ouabain, veratridine, batrachotoxin, and potassium ion should have considerable value in studies on transmitter release and the role of cyclic AMP in the central nervous system.

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