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EFFECTS OF CATECHOLAMINES ON CYCLIC-AMP LEVELS IN RAT SUPERIOR CERVICAL GANGLIA IN VITRO. H. Cramer*, D.G. Johnson*, S.D. Silberstein* and I.J. Kopin. NIMH, Bethesda, Md. 20014.

The effects of catecholamines on cyclic-AMP levels in superior cervical ganglia (SCG) of the rat were studied in vitro. Freshly dissected SCG of Sprague-Dawley rats (200 gram) were incubated in Krebs-Ringer solution at 37°C for various times with or without norepinephrine (NE), epinephrine (E), L-isonroterenol (ISO), L-phenylephrine (PHE) and dopamine (DA). Ganglia were homogenized in 6% trichloroacetic acid. The concentration of cyclic-AMP was determined according to A. Gilman (Proc. Nat. Acad. Sci. U.S.A. 67: 305, 1970). Cyclic-AMP levels were elevated rapidly during incubation of SCG with low concentrations (10^{-7}M to 10^{-6}M) of NE, E and ISO. Dose response curves were established for the amines. ISO (10^{-5}M) raised the cyclic-AMP concentration maximally to 800% of control values (1.3 pmoles/mg fresh weight) within fouur minutes. Higher concentrations of the amine were less effective. Preincubation for 12 minutes with propanolol (10^{-5}M) completely suppressed the rise of cyclic-AMP. PHE and DA had no effect on the cyclic-AMP levels. The findings are consistent with an activation of adenylcyclase by β-adrenergic agonists and suggest a role of cyclic-AMP in modulating activity in the SCG of the rat.

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CYCLIC AMP IN DISCRETE BRAIN REGIONS FOLLOWING MICROWAVE IRRADIATION. M.J. Schmidt* and G.A. Robison* (SPON: F. Sulser). Tennessee Neuropsychiatric Inst., Dept. of Pharmacol., Vanderbilt Univ. Sch. of Med., Nashville, Tennessee 37203.

Cyclic AMP levels in six regions of the brain were measured after exposure of rats to microwave irradiation (MI). The temperature of the brain was 50° C. following 5 seconds of MI and with continued exposure increased in a linear fashion reaching 90° C. within 30 seconds. Adenyl cyclase activity was reduced by more than 60% within 10 seconds of MI and was not detectable after 20 seconds. At this time phosphodiesterase was 95% inactivated. MI did not change the weight of the brain, but protein values were slightly lower in irradiated brains. Whole brain levels of cyclic AMP were 1.25 mμmoles/gm weight (12.2 μμmoles/mg protein) after 30 seconds of MI. The cyclic AMP concentration in discrete brain regions was highest in the cerebellum and brainstem, intermediate in the hypothalamus and midbrain, and lowest in the cortex and hippocampus. Following decapitation cyclic AMP levels increased in all areas of the brain, although the rise in the cerebellum was 2- to 3-fold higher than in other areas. Amphetamine did not produce a detectable change in the concentration of cyclic AMP in any of the 6 brain areas studied. (Supported by USPHS Grants AM-14240 and MH-11468).

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INCREASED SENSITIVITY TO NOREPINEPHRINE (NE) OF THE CYCLIC 3',5'-AMP (cAMP) SYSTEM OF RAT BRAIN FOLLOWING 6-HYDROXYDOPAMINE (6-HDM). S. J. Strada*, P. Uzunov*, and B. Weiss, Lab. of Preclinical Pharmacology, NIMH, St.Elizabeths Hosp.,Washington, D.C. 20032.

6-HDM produces a typical adrenergic denervation supersensitivity to NE (J. Pharmacol. exp. Ther. 170: 50, 1969). To determine if 6-HDM causes an increased sensitivity to NE of the cAMP system in the CNS, we studied adenylate cyclase (AC), cAMP and phosphodiesterase (PDE) in brains of rats given 6-HDM. Intraventricular or intracisternal injections of 6-HDM (250 μg at 0 and 48 hours; brains assayed 4 weeks later) did not alter the level of cAMP in brain stem or cerebrum nor modify the rise of cAMP in cerebellum after decapitation. However, after 6-HDM there was an increase in the levels of cAMP in incubated slices of brain stem and cerebellum and an increase in the NE-induced accumulation of cAMP in slices of brain stem and cerebrum. These findings may be explained by an inhibition of PDE or by an increase in AC. PDE was not altered in any brain area studied. However, after 6-HDM treatment, responsiveness of the pineal AC to NE was greater. Thus, the increased level of cAMP in incubated brain slices and the greater accumulation of cAMP induced by NE after 6-HDM treatment may be due to an increased sensitivity of the brain AC system to catecholamines.

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EFFECT OF PSYCHOTOMIMETIC DRUGS ON THE CYCLIC 3',5'-AMP SYSTEM OF RAT BRAIN. P. Uzunov* and B. Weiss, Lab. of Preclinical Pharmacol.,NIMH, St. Elizabeths Hosp., Washington,D.C. 20032.

Previous studies showing that phenothiazine tranquilizers inhibit the activation of the cyclic AMP system of rat brain suggested that the cyclic nucleotide may play a role in the pathogenesis of certain psychoses. To study this possibility further, we have examined the effects of several structurally dissimilar psychotomimetic agents on the cyclic AMP system of rat brain. All psychotomimetics studied, including d-LSD, mescaline, psilocybin, ibotenic acid and the dimethyl, diethyl, and dipropyl derivatives of tryptamine (0.5 mM) increased the accumulation of cyclic AMP when added in vitro to brain tissue slices. The inactive metabolites of these agents (e.g. L-LSD, bromo LSD) had little or no effect on the concentration of cyclic AMP. Addition of trifluoperazine (TFP) (0.5 mM) to the tissue slices or administering it to the rat (50 μmoles/kg i.p.) 90 minutes before preparing the slices prevented the accumulation of cyclic AMP induced by the psychotomimetics. The in vivo administration of d-LSD (1 mg/kg i.p.) raised the concentration of cyclic AMP in cerebrum two-fold within 10 minutes after the injection. TFP (50 μmoles/kg i.p.) given 60 minutes before LSD totally abolished this effect. These results support the concept that cyclic AMP may be involved in experimental psychoses.

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KINETIC CONSEQUENCES OF INHIBITION OF CYCLIC 3',5' NUCLEOTIDE PHOSPHODIESTERASE. R. P. Miech, Div. Biol. & Med. Sciences, Brown University, Providence, R. I. 02912

The final biochemical effect of cyclic 3',5' AMP is in some cases mediated through a sequential array of enzymatic reactions in which the product of one reaction serves as the enzyme in the next, except for the last step in which the product is not an enzyme. Sequential enzymatic activation of the next enzyme in a series results in an enzyme cascade system (avalanche effect or multi-stage amplifier). Kinetic equations were derived for cyclic 3',5' AMP activation of a multi-enzyme cascade system. In addition to biochemical amplification and fast response time, these equations indicate a key role of cyclic nucleotide phosphodiesterase in regulating and modulating the final biochemical event of this cascade system. Two possible mechanisms exist whereby the intracellular level of 3', 5' cyclic AMP may be increased: the activation (stimulation)of adenylcyclase by hormones or the inhibition of cyclic 3',5' nucleotide phosphodiesterase by drugs. However, the degree of biochemical amplification and the time course for the rise in cyclic 3',5' AMP concentration compared with the time for the final biochemical event are markedly different depending upon whether the increase in cyclic 3',5' AMP is due to increased adenylcyclase activity or inhibition of cyclic 3',5' nucleotide phosphodiesterase. Supported by USPHS, NS-06034.

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INHIBITION BY VINCA ALKALOIDS AND PAPAVERINE OF THE EXTRUSION OF CYCLIC AMP FROM PIGEON ERYTHROCYTES. Carl D. King* and Steven E. Mayer. Univ. Cal. San Diego; La Jolla, Cal. 92037.

As first noted by Davoren and Sutherland (1963), pigeon erythrocytes possess a catecholamine-sensitive adenylate cyclase system; the cyclic AMP (CA) which is formed is then extruded from the cells against a concentration gradient. We have found that vinblastine (VBL) and vincristine (VCR) inhibit the extrusion process following stimulation of cyclic AMP production by 10^{-5}M norepinephrine. Inhibition is complete for both alkaloids when their concentration is 10^{-4}M. Inhibition is also seen with concentrations as low as 10^{-6}M. The two alkaloids are about equally potent. Papaverine (P) markedly augments the amount of CA formed after catecholamine stimulation. At 10^{-4}M, P sharply inhibits the extrusion of CA from the cells into the medium. At 10^{-5}M, P alone still augments catecholamine-induced production of CA, but no longer inhibits the extrusion of CA from the cells. These data provide additional evidence that the vinca alkaloids can inhibit processes whereby products are extruded outwards from the interior of the cell. The data also suggest that high concentrations of P augment intracellular levels of CA both by inhibition of phosphodiesterase and by inhibition of the egress of CA from within the cell. (Supported by grant HE-12373 and fellowship HE-30804 from the National Heart and Lung Institute.)