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Dopamine and Macromolecule Synthesis in Rat Hippocampus[1]

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JORK, R., B. LÖSSNER AND H. MATTHIES. *Dopamine and macromolecule synthesis in rat hippocampus.* PHARMAC. BIOCHEM. BEHAV. 11(2) 247–249, 1979.—In hippocampus slices both dopamine and apomorphine lead to an increased incorporation of (^{3}H)-fucose into total proteins, whereas the incorporation of (^{14}C)-leucine was unchanged or decreased, respectively. Noradrenaline did not alter the incorporation of both precursors, whereas haloperidol partially reduced the dopamine induced increase in incorporation of fucose. Thus, an induction process of the observed macromolecular changes involving dopaminoceptive structures of hippocampus can be assumed.

Dopamine (^{3}H)-Fucose (^{14}C)-Leucine Hippocampus

NUMEROUS biochemical investigations indicate changes in the incorporation of precursors into proteins and glycoproteins of rat hippocampus related to training experiments and simple neuronal activation, respectively [5, 6, 11, 12, 14, 15]. On the other hand recent studies have provided evidence that changes in the incorporation of macromolecule precursors can be elicited by interactions between transmitters and receptors or coupled biochemical events [3, 4, 7, 10]. One can speculate that the induction of complex macromolecular changes in the hippocampal structure during learning should be due to a spatial temporal pattern of afferent neuronal inputs that might be mediated by different transmitter systems. Therefore the question arose as to whether some transmitter systems of the hippocampus are responsible for the observed changes in macromolecular metabolism. Thus, the influence of some transmitter substances on incorporation of leucine and fucose into macromolecules of hippocampal slices was determined. First results showed that neither muscarinergic nor nicotinergic cholinergic agonists alter the incorporation of both precursors in such a way seen in learning experiments and simple neuronal activation, respectively [9]. Therefore catecholamines were investigated in this field. Though the sensitivity of the hitherto existing biochemical methods did not give proof for the occurrence of dopamine as a transmitter in the hippocampus, the local application of dopaminergic agonists into the dorsal hippocampus do induce a slow rhythmic activity as well as lead to an improvement of the retention of a footshock motivated brightness discrimination in rats, an effect which can be abolished by haloperidol [9]. Thus, it seemed worthwhile to investigate the influence of dopamine on the incorporation of leucine and fucose into macromolecules of hippocampal slices in vitro.

METHOD

For the experiments, eight-week-old male Wistar rats from our own breeding stock were used. From the removed hippocampus [13] 0.5 mm thick slices were prepared by cutting the structure perpendicularly to its longitudinal axis [16]. In each case three slices of one hippocampus were used as controls and for testing catecholaminergic substances, respectively. After a preincubation period of 15 min in 0.8 ml incubation medium (134 mM NaCl; 16 mM $NaHCO_3$; 0.75 mM $CaCl_2$; 1.24 mM KH_2PO_4; 1.3 mM $MgSO_4$ and 10 mM glucose, pH 7.35) the substances studied were added to the incubation medium at a volume of 100 μl. The medium of the control slices was refilled to a total amount of 0.9 ml. Ten min later 25 μCi L-(1-^{3}H)-fucose (specific activity 2 Ci/mmole, Radiochemical Centre, Amersham, Great Britain) and 3 μCi L-(U-^{14}C)-leucine (specific activity 185 mCi/mmole, Prague, Czechoslovakia) were added to control and transmitter treated slices at a volume of 100 μl. The concentration of the precursors in the incubation medium was 10^{-3} M [1]. Throughout the experiment the slices were treated with carbogen (95% O_2, 5% CO_2); the incorporation time being 60 min. After homogenization of the tissue in 0.3 ml 0.1 N NaOH, from 0.2 ml of the solution the total proteins were precipitated using 0.5 ml ice-cold 12% trichloracetic acid. The precipitate was washed twice with 6% trichloracetic acid; water; ethanol-ether (1:1, v:v) and dissolved in Hyamine hydroxide (New England Nuclear). The samples were dissolved in a dioxane containing scintillator and their radioactivity was determined in a liquid scintillation spectrometer (Intertechnique, Plaisir, France). From the obtained values of radioactivity and protein content of homogenate [8] the specific activity of proteins (d.p.m./mg protein) was calcu-

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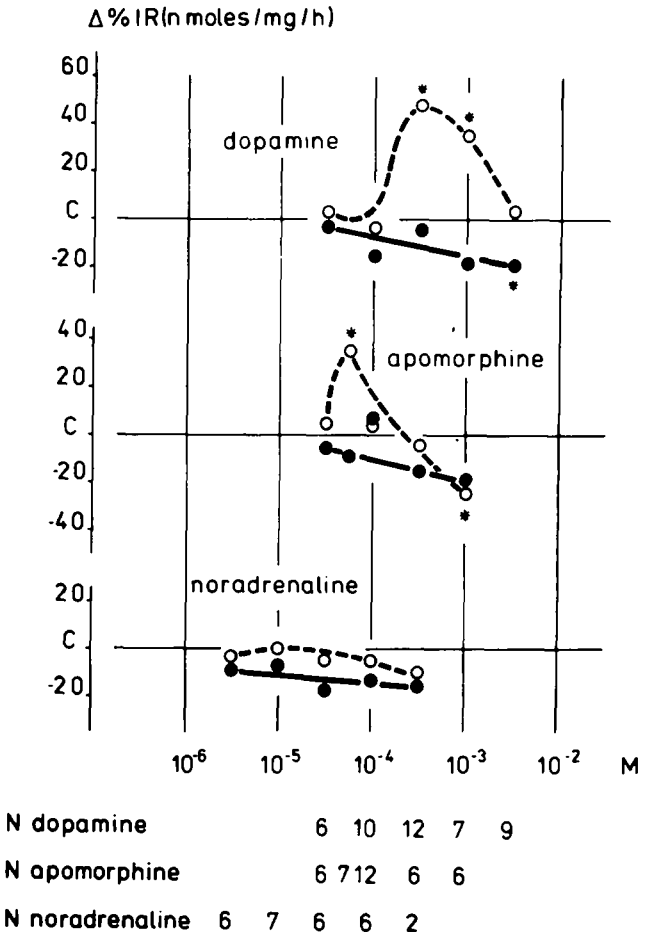

FIG. 1. Mean value of the percentage deviation of the incorporation rate IR (nmoles/mg protein/hour) of the precursors (^{3}H)-fucose (○---○) and (^{14}C)-leucine (●———●) from control level (C) under the influence of dopamine, apomorphine and noradrenaline, respectively. *$p<0.05$ according to Wilcoxon matched pairs signed rank test.

lated. For calculation of the incorporation rate IR (nmoles/mg protein/hour) the specific activity of proteins was corrected by the amount of radioactive precursor of incubation medium using the following equation:

$$IR = \frac{\text{d.p.m.} \times \text{mg protein}^{-1} \times \text{h}^{-1}}{\text{d.p.m.} \times \text{nmoles precursor}^{-1}_{(\text{medium})}}$$

This calculation is based on the consideration that within a few minutes the specific activity (d.p.m.×nmoles precursor^{-1}) of the intracellular space will be identical with that of the medium [1].

RESULTS AND DISCUSSION

In the first series of experiments the influence of different concentrations of dopamine on incorporation of (^{3}H)-fucose and (^{14}C)-leucine into hippocampal proteins was investigated. As shown in Fig. 1 dopamine leads to a significant increase in the incorporation of (^{3}H)-fucose whereas the incorporation of (^{14}C)-leucine was unchanged or decreased, respectively. The question arose as to whether these changes could be due to noradrenaline build up from the added dopamine. Thus, the influence of the dopaminergic agonist apomorphine as well as the influence of noradrenaline on precursor incorporation was tested. As shown in Fig. 1 apomorphine induces the same alterations in incorporation of both precursors seen by dopamine, whereas noradrenaline does not alter the incorporation of (^{3}H)-fucose and (^{14}C)-leucine at all. The fact that the changes induced by apomorphine occur at a lower concentration in comparison to dopamine is in good agreement with the higher affinity of apomorphine to the dopamine receptor [2]. To get some more hints for the specificity of the observed changes in macromolecular metabolism haloperidol was investigated in order to inhibit the increase in the incorporation of fucose under the influence of dopamine. As shown in Fig. 2 haloperidol partially but significantly reduced the incorporation of (^{3}H)-fucose induced by dopamine. Summarizing the results, it can be assumed that dopamine and apomorphine lead to an increased incorporation of (^{3}H)-fucose into hippocampal glycoproteins by acting with dopaminoceptive

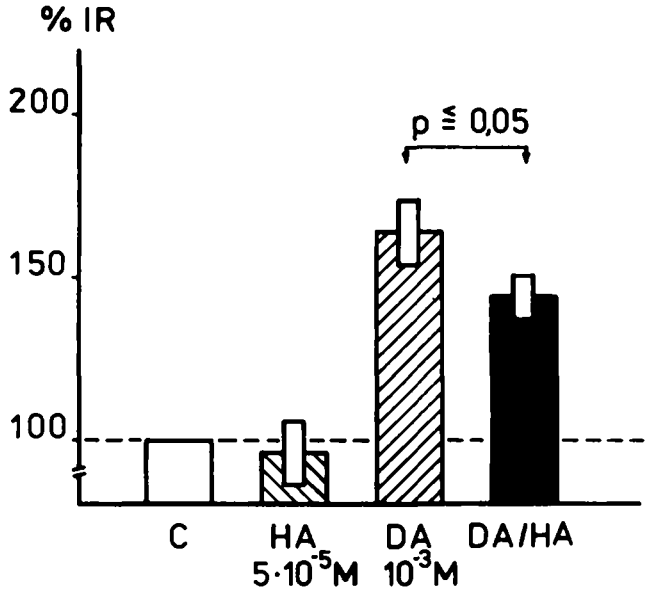

FIG. 2. Mean value of the percentage deviation of the incorporation rate IR (nmoles/mg protein/hour) of (^{3}H)-fucose from control level (C) under the influence of haloperidol (HA), dopamine (DA) and a mixture of both. In these experiments the incorporation rate of (^{3}H)-fucose of controls was 0.41 nmoles/mg/h (n=9). Statistical significance is based on Wilcoxon matched pairs signed rank test.

structures of these brain regions. These changes can be partially blocked by haloperidol, a well known antagonist of the dopamine receptor. Preliminary results have shown that dibutyryl-cAMP leads to an increased incorporation of (^{3}H)-fucose into glycoproteins of hippocampal slices as well, referring to the importance of a second messenger system for the induction of the observed macromolecular changes. Further investigations are required to ascertain the physiological significance of dopamineceptive structures in the hippocampus of rats.

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