Pharmacology Biochemistry & Behavior, Vol. 11, pp. 243–245. Printed in the U.S.A.

The Influence of Cholinergic Transmitter Substances on the Incorporation of (^{14}C)-Leucine and (^{3}H)-Fucose into the Total Proteins of Hippocampus in vivo and in vitro[1]

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Received 20 April 1979

JORK, R., B. LÖSSNER AND H. MATTHIES. *The influence of cholinergic transmitter substances on the incorporation of (^{14}C)-leucine and (^{3}H)-fucose into the total proteins of hippocampus in vivo and in vitro.* PHARMAC. BIOCHEM. BEHAV. 11(2)243–245, 1979.—The incorporation of (^{14}C)-leucine into the total proteins of the hippocampus is inhibited by high concentrations of cholinergic agonists, with nicotinic substances (such as 1,1-dimethyl-4-phenyl-piperazine) being more effective than muscarinic compounds (such as arecoline and pilocarpine). Under these conditions the incorporation of (^{3}H)-fucose is not influenced.

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

RECENT studies have provided evidence that after acquisition of a brightness discrimination in rats the incorporation of both (^{3}H)-leucine and (^{3}H)-fucose into proteins of the hippocampus was significantly increased [14], whereas the induction of hippocampal theta rhythm by electrical stimulation of the medial septum led only to an enhanced incorporation of (^{3}H)-leucine into the total proteins of distinct hippocampal sectors, whereas the incorporation of (^{3}H)-fucose was not influenced [8]. Changes in incorporation of precursors of some macromolecules due to electrical stimulation of neurons are supposed to be the result of interactions between transmitters and receptors or coupled biochemical events rather than being attributed to electrophysiological membrane phenomena [6, 7, 9, 15]. Considerable experimental evidence indicates that the monosynaptic nerve fibres running from the septum to the hippocampus are of a cholinergic nature and the electrical stimulation of these fibres causes an increased release of acetylcholine in the hippocampus [4, 10, 18]. In view of these considerations, the present study was undertaken to ascertain if the proved enhancement in (^{3}H)-leucine incorporation into hippocampal proteins upon application of electrical stimulation of the septum is implemented by the interaction between the transmitter acetylcholine and the cholinergic receptors. Furthermore, the study attempted to test if the (^{3}H)-fucose incorporation into glycoproteins can be influenced by cholinomimetics.

METHOD

The experiments were performed on eight-week-old male Wistar rats of our own breeding stock. In the case of in vitro studies after decapitation the removed hippocampi [17] were cut perpendicularly to the longitudinal axis using a cutter [21] to give slices of 0.5 mm thickness. In each case three hippocampal slices were preincubated in 0.8 ml of a medium containing 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 at pH 7.35 for 15 min. Afterwards the transmitter substance dissolved in 100 μl was added to the incubation medium; the incubation medium of the control slices was refilled to a total amount of 0.9 ml. Ten minutes later, 100 μl solution containing 25 μCi L-(1-^{3}H)-fucose (specific activity 2 Ci/mmole, purchased from the 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. In each case the concentration of the precursors in the incubation medium was 10^{-3} M [5]. The tissue slices were aerated with carbogen (95% O_2 and 5% CO_2) throughout the experiment. After an incorporation time of 60 minutes the slices were removed from the incubation medium and frozen on dry ice. The tissues were then homogenized in 0.3 ml of 0.1 N NaOH; 0.2 ml of this solution were treated with 0.5 ml ice-cold 12% trichloroacetic acid to precipitate the total proteins. The precipitate was washed twice with 6% trichloracetic acid;

[1]This research was supported by the Ministry of Science and Technology of the G.D.R.

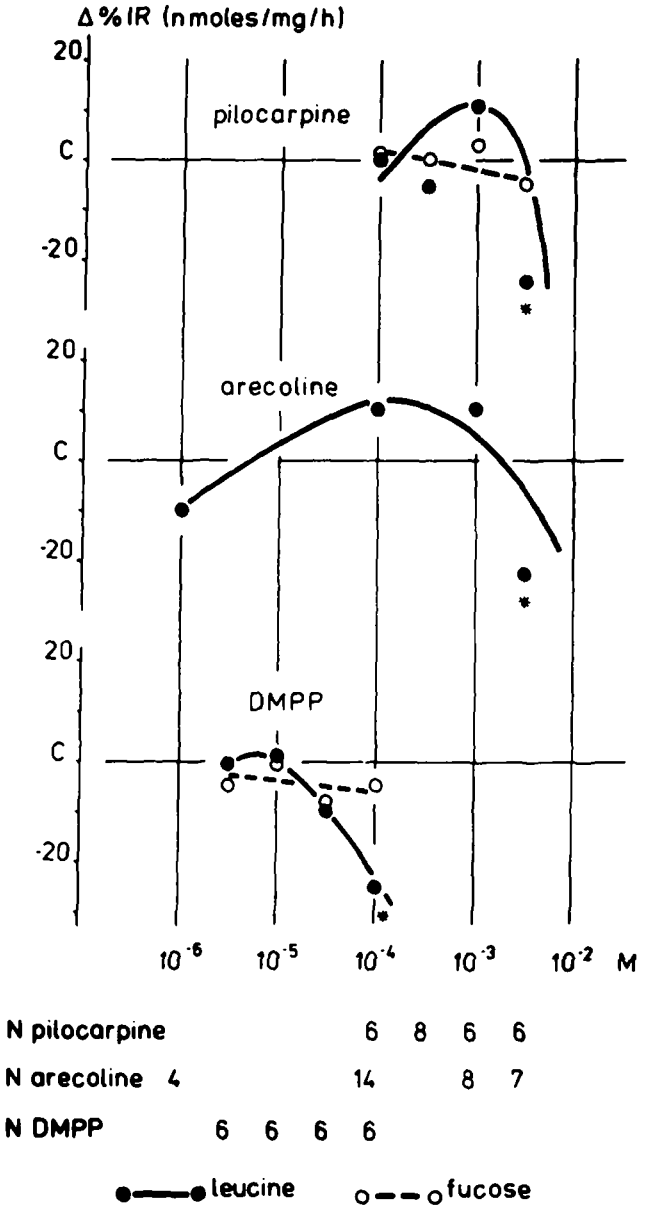

FIG. 1. Mean value of the percentage deviation of the incorporation rate IR (nmoles/mg protein/hour) of the precursors [^{3}H]-fucose (○---○) and (^{14}C)-leucine (●——●) from control level (C) under the influence of different cholinergic agonists using hippocampus slice system in vitro. Under the chosen experimental conditions for control slices the incorporation rates for (^{14}C)-leucine and (^{3}H)-fucose were 0.29 nmoles/mg/h and 0.37 nmoles/mg/h, respectively. *$p<0.05$ according to Wilcoxon matched pairs signed rank test [22].

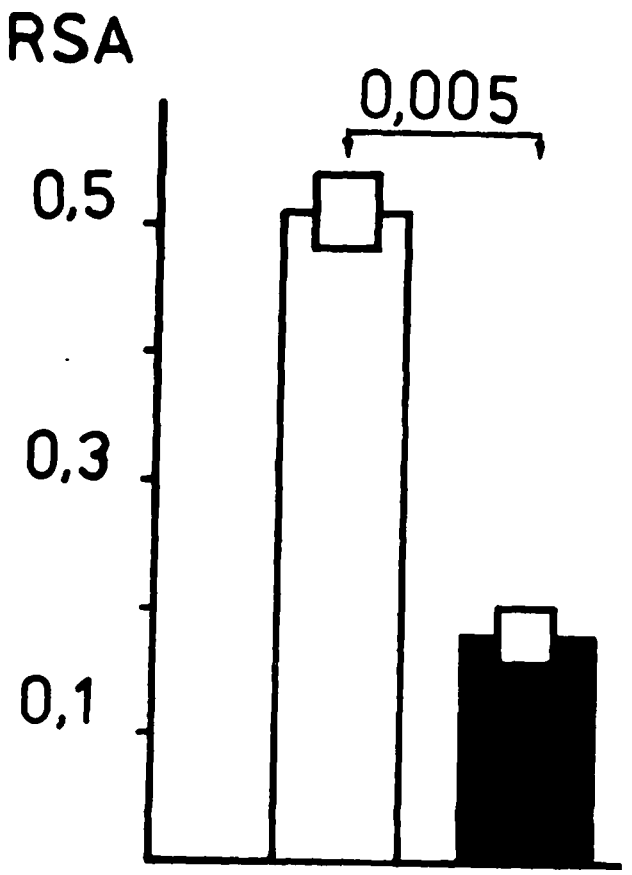

FIG. 2. Incorporation of (^{3}H)-leucine, represented as relative specific activity of proteins (RSA) in the rat dorsal hippocampus under control conditions (□, N=5) and after topic application of 4.75 μg arecoline hydrobromide (■, N=5) given by the mean values Mean ± S.E.M. of both experimental groups. Statistical evaluation of the experimental findings was performed using the U-test of Mann and Whitney [22].

water; ethanol—ether (1:1, v:v) and dissolved in Hyamine hydroxide (New England Nuclear). The samples were soluted in 14 ml of a dioxane scintillator solution (6 g PPO, 0.2 g POPOP, 100 g naphthalene, distilled dioxane at 1000 ml) and the radioactivity was measured on a MULTIMAT Scintillation Spectrometer (Intertechnique, Plaisir, France). The protein content of homogenate samples was determined using the method described by Lowry *et al.* [13]. The incorporation rate IR (nmoles/mg protein/hour) of the precursors was calculated using the following equation

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

based on the consideration that within a few minutes the specific activity (d.p.m. × nmoles precursor^{-1}) of the intracellular space is identical with that of the medium [5]. In order to exclude the possibility that the observed decrease in (^{14}C)-leucine incorporation would depend on the conditions of the in vitro system used, the influence of a high arecoline concentration on amino acid incorporation was tested in a further experiment in vivo. For this purpose 1 μl of arecoline solution (artificial cerebrospinal fluid containing 4.75 μg arecoline hydrobromide) was injected into the dorsal hippocampus through a chronically implanted cannula (AP -3.1 mm; lateral 3.1 mm; vertical 3.1 mm); the control animals received an identical volume of artificial cerebrospinal fluid. Five minutes later, 10 μCi L-(4,5-^{3}H)-leucine (specific activity 58 Ci/mmole; Radiochemical Centre, Amersham, Great Britain) dissolved in 1 μl artificial cerebrospinal fluid was applied. The incorporation time was 15 min. After homogen-

ization of the tissue, total proteins were precipitated and washed as described. The incorporation rate was expressed as relative specific activity of proteins (RSA), i.e. the ratio of the specific activity of proteins to the radioactivity of the free, non-incorporated precursor, separated from the trichloracetic acid soluble supernatant by DOWEX ion exchanger (2 × 8; 200–400 mesh; SERVA, Entwicklungslabor Heidelberg).

RESULTS AND DISCUSSION

Taking into consideration that the cholinergic receptors of the hippocampus are predominantly of muscarinic nature [1, 2, 3, 11, 20], in a first series the influence of the muscarinic agonist pilocarpine (pilocarpine hydrochloride) on the incorporation of (^{14}C)-leucine and (^{3}H)-fucose was studied, As demonstrated in Fig. 1, pilocarpine at all concentrations applied exerted no influence on (^{3}H)-fucose incorporation into hippocampal glycoproteins. Likewise the incorporation of (^{14}C)-leucine into the total proteins showed no statistically significant increases; only high concentrations of the agonist caused a pronounced decrease in incorporation. Analogous findings were obtained when the action of arecoline hydrobromide was studied in vitro and in vivo (Fig. 1 and 2). Despite the prevelance of muscarinic cholinergic receptors in the hippocampus it seems that also the nicotinic cholinergic receptors [16,19] as well as the interaction between both kinds of receptors [1] are meaningful.

On this background the influence of the nicotinic agonist 1,1-dimethyl-4-phenyl-piperazine iodide (DMPP) on the incorporation of (^{14}C)-leucine and (^{3}H)-fucose was tested. As shown in Fig. 1, the incorporation of the precursors was influenced in a manner similar to the experiments with muscarinic agonists. Thus, the incorporation of (^{3}H)-fucose remained uninfluenced, whereas after application of increasing concentrations of DMPP the (^{14}C)-leucine incorporation was decreased. However, it must be noted in comparison to the results obtained with muscarinic agonists that this effect was evident even after application of lower concentrations. This finding could suggest that the effect of muscarinic agonists is due to the nicotinic component which, although very small, may become effective at the very high concentrations chosen. Nevertheless under our experimental conditions neither muscarinic nor nicotinic agonists exerted an unambiguous increase in incorporation of (^{14}C)-leucine into total proteins of the hippocampus. This finding could be interpreted in terms that the proved increase in incorporation of the amino acid after activation of hippocampal neurons by septal stimulation [8] is not primarily the sequel of an interaction of the enhanced release of the transmitter acetylcholine with the cholinergic receptors, but may be attributed to the effect of other transmitters. Thus, the influence of other transmitter systems on synthesis of proteins and glycoproteins will be studied in further investigations.

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