taurine may be a factor controlling both the synthesis and catabolism of cyclic nucleotides [1]. Whatever its nature, the inhibitory action of taurine on the formation of cyclic AMP and cyclic GMP under conditions of stress can be regarded as a protective response of the body against overloading with adrenalin and ACh under extremal conditions.

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CHANGES IN ACTIVE UPTAKE OF NORADRENALIN-^{14}C BY RAT BRAIN SYNAPTOSOMES DURING CONDITIONING

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Active uptake of noradrenalin-^{14}C (NA-^{14}C) by rat brain synaptosomes was shown to be inhibited by the action of ouabain (0.1 M) and during potassium depolarization. Defensive conditioning (DC) led to depression of active uptake of NA by synaptosomes. This effect may be connected both with changes in the state of the presynaptic membranes and with possible enhancement of the function of postsynaptic adrenergic receptors.

KEY WORDS: conditioning; active uptake of noradrenalin; rat brain synaptosomes.

An important step in the explanation of the mechanisms of formation and fixation of temporary connections is the study of functional changes in the synapses which may give rise to changes in their structure. A neurochemical index of a change in the efficacy of synaptic transmission is the highly specific active uptake of mediators by nerve endings [1, 9]. This index largely reflects presynaptic mechanisms of regulation of synaptic activity, namely the reassimilation of mediators, which is a method of inactivation of neurotransmitters and which probably plays an essential role in the realization of synaptic functions.

The basis for this investigation consisted of research which showed that, under certain conditions (stress [7, 8], electric shock [4]) the rate of uptake of biogenic amines by the corresponding nerve endings is changed, as experiments in vitro with labeled mediators have shown.

The object of this investigation was to study active uptake of noradrenalin-^{14}C (NA-^{14}C) by isolated nerve endings (synaptosomes) during defensive conditioning (DC).

EXPERIMENTAL METHOD

Experiments were carried out on 68 noninbred male albino rats weighing 180-200 g. Defensive conditioning to electric shock was produced in the animals in a shuttle chamber, in the form of bilateral avoidance with a 5-sec delay. The photic stimulus was light. Five correct responses to six presentations served as the criterion of DC.

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TABLE 1. Uptake of Labeled NA (10^{-5} M) by Rat Brain Synaptosomes (500 μg protein/ml; M ± m)

Experimental conditions	Rate of uptake, cpm/mg protein	Change in rate of uptake, %	Number of animals	Passive control
Control (37°C)	31875,3±1378,0	100,0	30	10
Incubation (0°C)	2008,3±112,6	6,3*	30	10
Ouabain (0.1 M)	19125,2±837,2	60,0*	21	7
K-depolarization (60 mM KCl)	20145,3±1029,8	63,2*	18	6

*$P<0,05$.

The animals were divided into two groups. Defensive conditioning took 7-10 min in one group and 18-20 min in the other. Animals which received the same number of conditioned and unconditioned stimuli as the experimental animals within the same time interval were selected individually to each experimental animal (active control). Intact animals served as the passive control. Immediately after these procedures the animals were decapitated, the brain was extracted, and the cerebellum was removed and placed in liquid nitrogen. After thawing of the tissue synaptosomes were isolated [6].

The brain tissue was homogenized in 18 ml of 0.3 M sucrose solution and centrifuged (1500g, 10 min). The supernatant was centrifuged (9000g, 25 min) and the residue was resuspended in 5 ml 0.3 M sucrose, layered above a layer of 0.8M sucrose (20 ml), and centrifuged for 20 min at 9000g. The suspension of synaptosomes in the layer of 0.8 M sucrose was then separated and diluted with 0.1 M sucrose to a concentration of 0.3 M. Synaptosomes were obtained as a residue by centrifugation (20,000g, 30 min, K-24 centrifuge — from East Germany). The residue was resuspended in cold Krebs — Ringer solution, containing (in mM) NaCl 104, KCl 5, $MgCl_2$ 1.3, NaH_2PO_4 1.2, glucose 10, Tris-HCl, pH 7.4, 20, and $CaCl_2$ 1.2. The protein concentration was determined by Lowry's method. Samples (250 and 500 μg protein/ml) were kept for 10 min at 0°C, then preincubated (10 min, 37°C), and incubated for 10 min at 37°C in the presence of $7.0 \cdot 10^{-6}$ or 10^{-5} M NA-^{14}C (specific radioactivity 35 mCi/mmole, Radiochemical Centre, Amersham, England). After incubation, the samples (1 ml) were diluted fourfold with Krebs — Ringer solution and centrifuged (20,000g, 5 min, 0-4°C). The residue was resuspended several times, washed, dried, and then dissolved in 0.5 ml of 1 N NaOH (45°C); 0.1 ml of the solution was transferred to a counting flask containing a mixture of scintillation fluid (4 g PPO, 0.1 g POPOP, 1 liter toluene) with ethanol (7:3). Radioactivity was counted in a scintillation counter (SL-30, Intertechnique, France). In all cases control checks were made for adsorption of label, which averaged 25% of the total NA binding. The results were subjected to statistical analysis by means of Student's criterion.

EXPERIMENTAL RESULTS

The isolated rat brain synaptosomes contained minimal contamination — not more than 5-8% of mitochondria and myelin.

It will be clear from Table 1 that the uptake of labeled NA (10^{-5} M) by synaptosomes is a highly selective and active process, for the rate of uptake is reduced practically to zero at 0°C and considerably reduced by various depolarizing procedures. Active uptake of NA by nerve endings against the concentration gradient is observed in a concentration of 10^{-5}-10^{-7} M of mediator in the incubation medium [9]. The actively assimilated NA is mainly stored in the synaptic vesicles, whereas passively assimilated NA is metabolized [3]. Active uptake of NA is closely linked with the function of the Na,K-pump and, for that reason, the process can be inhibited by ouabain. Both ouabain and other depolarizing substances also are known to inhibit the active uptake of mediators, especially NA [1].

Considering that in these experiments neither ouabain nor K-depolarization completely inhibited the uptake of NA by synaptosomes (10^{-5} M), in the next experiments with DC a lower concentration of NA was used in order to study only active uptake without the passive components.

As Table 2 shows, significant changes in the rate of uptake of NA by the synaptosomes during DC were observed only if NA was present in a concentration of $7 \cdot 10^{-6}$ M and synaptosomes in a concentration of 250 μg protein/ml. As a result of DC the active uptake of NA was inhibited by 20% relative to both control groups; the error of the method, i.e., scatter between the samples in one experiment, was 1-2%. Meanwhile no significant difference was observed between the active and passive control groups. The results for the two groups of rats — those fast and slow to learn — are summarized in Table 2. The results were pooled because no difference was found between these groups as regards either the direction or the intensity of changes.

TABLE 2. Effect of DC and Uncombined Presentations of Stimuli on Active NA Uptake by Rat Brain Synaptosomes (M ± m)

Experimental conditions	500 μg protein/ml; 10^{-5} M NA				250 μg protein/ml; $7 \cdot 10^{-6}$ M NA			
	rate of uptake, cpm/mg protein	change in rate of uptake, %	No. of tests	No. of animals	rate of uptake, cpm/mg protein	change in rate of uptake, %	No. of tests	No. of animals
Passsive control	35143,6±1629,3	100,0	15	5	43104,6±1103,4	100.0	56	7
Active	34159,6±1577,4	97,2	15	5	43492,5±1823,1	101.0	48	6
DC	37159,6±1938,6	107,0	15	5	34311,3±1107,7	79,6*†	56	7

* $P<0,001$ compared with passive control.
† $P<0,01$ compared with active control.

The process of active uptake of NA by nerve endings consists of a mechanism of inactivation of the NA after its liberation into the synaptic space. Consequently, DC causes inhibition of the inactivation process. Such inhibition can increase the action of the mediator on postsynaptic structures. The system of NA uptake can be controlled both pre- and postsynaptically. The latter possibility is indicated by data [2] showing that activation of postsynaptic adrenoreceptors leads to a decrease in active NA uptake. These factors must be taken into account when the results of the present experiments are explained.

The results are of special importance because they were observed during experiments in vitro. This fact indicates that activity of synaptic structures, modified by the formation of a temporary connection, is preserved after conditioning. The decrease in active uptake of NA during conditioning evidently does not reflect any adverse action on the restoration of its reserves in nerve endings. During liberation of catecholamines from nerve endings the mechanism of their more rapid biosynthesis is activated. Under these circumstances, a change in their concentration in the synapses has a modulating effect on catecholamine synthesis [5].

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