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Alteration of Cyclic Nucleotide Levels in Brain Following Intracranial Self-Stimulation in the Rat[1]

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MILIARESSIS, E. AND Z. MERALI. *Alteration of cyclic nucleotide levels in brain following intracranial self-stimulation in the rat.* PHARMAC. BIOCHEM. BEHAV. 11(1) 107–110, 1979.—In a first experiment, 14 rats were implanted with an electrode in the ventral tegmental area and trained to self-stimulate. On the experimental day only half of the rats were allowed to self-stimulate for one hour. All rats were then sacrificed by immersion in liquid nitrogen. Seven brain regions were dissected and assayed for the endogenous concentration of cyclic nucleotides. Self-stimulation induced significant changes in striatum and hippocampus. However, a subsequent experiment showed that the same pattern of changes in the striatum can be produced by motor activity. On the other hand, changes in the hippocampus were specific to the self-stimulation group suggesting that this structure is associated with the brain reward system.

Self-stimulation Motor activity Cyclic-nucleotides Striatum Hippocampus

THE purpose of the present work was to investigate possible changes in the endogeneous brain concentrations of cyclic-adenosine monophosphate (c-AMP) and cyclic-gyanylyl monophosphate (c-GMP) following intracranial self-stimulation (ICSS) in the rat.

Cyclic-AMP and c-GMP are normal constituents of various types of somatic cells [28] and of central neurons [6,18]. Their synthesis is catalyzed by the enzymes adenylate cyclase and gyanylyl cyclase, respectively [28]. In the CNS, it was shown that adenylate cyclase can be stimulated by the putative neuro-transmitters catecholamines, serotonin and histamine [12,23]. Presently it is thought that adenylate cyclase is intimately associated with the post-synaptic catecholaminergic receptor, and that fluctuations in c-AMP levels may be involved in the transmission of neural information [28]. The physiological role of c-GMP is presently unknown, though its levels seem to change in an opposite direction to those of c-AMP [26].

Since positive reward is believed to be dependent on the release of catecholamines [7], and possibly serotonin [13,31], ICSS could be expected to modify the endogeneous concentration of c-AMP.

The present experiments were then undertaken in an attempt to elucidate whether ICSS is accompanied by alterations in central c-AMP and c-GMP content, and to establish whether these changes are localized to the brain regions specifically associated with positive reward.

METHOD

Animals and Surgery

In a first experiment, 14 male Sprague-Dawley rats (280 g) were stereotaxically implanted under general anaesthesia (nembutal 40 mg/kg IP) with one bipolar electrode in the A10 dopaminergic cell group of Dahlström and Fuxe [5] which lies in the ventral tegmental area (VTA). With the incisor bar 5 mm below the interaural line, coordinates of implantations were as follows: 5.3 mm posterior to the bregma, 0.4 mm lateral to the midline and 8.2 mm under the surface of the skull.

Procedure

Following one week of post-operative rest, animals were trained to press a lever in a skinner box in order to stimulate their brain at the tip of the electrode. Each lever press was immediately followed by a 200 msec train of 60 c/sec sin. current. The intensity of the stimulation was individually

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adjusted so that optimal self-stimulation frequency was obtained by each rat. Following 10 daily 60 min ICSS periods, all but two rats were placed in the experimental boxes for the final 60 min session, but only half of them, (randomly chosen) were delivered the 200 msec train of current, upon lever pressing. At the end of the 60 min period, self-stimulating and control rats were killed by immersion into liquid nitrogen, according to the *near freezing* technique of Takanashi and Aprison [29]. Brains were then removed and seven regions dissected and stored frozen until time of essay. Cyclic AMP and cyclic GMP were measured by the radioimmunoassay of Steiner *et al.* [26] using commercially available antibodies and labelled nucleotides. The antigens were acetylated by the procedure of Harper and Brooker [9]. The brain of the remaining two animals were kept in 10% Formalin. Brain section (30 μ thick) were mounted on slides for macroscopic examination of electrode placements.

Figure 1 shows drawings of brain slides containing the electrodes of two rats randomly chosen among the 14 self-stimulating animals. As it can be seen, the tips of the electrodes were located in the VTA, just above the A10 cell group, dorsolaterally to the interpedoncular nucleus. It is now well established that electrodes in this area sustain very high rates of ICSS [14]. In the last 60 min training period of the present experiment, control and experimental rats performed a total of 4264 ± 526 and 4036 ± 486 lever presses with 80 ± 9 and 84 ± 11 microamperes, respectively.

As it can be seen in Table 1, self-stimulation failed to alter significantly the concentration of c-AMP and c-GMP in five out of the seven brain regions, (cortex, hypothalamus, olfactory bulb, pons and medulla). On the other hand, significant changes were observed in the striatum and hippocampus. Two interesting observations are that in the above two regions the effects of ICSS are opposite and that c-AMP and c-GMP vary in opposite directions. From the present results, it seems possible that changes in striatum and hippocampus are associated to rewarding neuronal activity induced by VTA stimulation. It should be noted, however, that these changes could also be attributable to the motor activity which necessarily accompanies ICSS. In order to examine this contention, the effects of motor activity on c-AMP and c-GMP concentrations were examined in the following experiment.

Animals and Procedure

Twelve rats (300 g at time of sacrifice) were allowed to perform 10–30 min daily sessions of motor exploration in a large open-field box. On the eleventh day, only half of the animals, randomly chosen (experimental group) were allowed to explore in the box while the remaining animals (control group) were kept in their home cages. At the end of the open-field test, experimental and control rats were alternatively sacrificed and brain regions frozen, and analyzed as described earlier.

RESULTS AND GENERAL DISCUSSION

Table 2 shows values of c-AMP and c-GMP concentrations of six brain regions of experimental and control rats. Comparison with Table 1 shows remarkably similar values between control rats of Experiment 1 and 2 in all regions but one (olf. bulbs). Consequently, an artifactual biochemical explanation of the data obtained in the second experiment can be rejected.

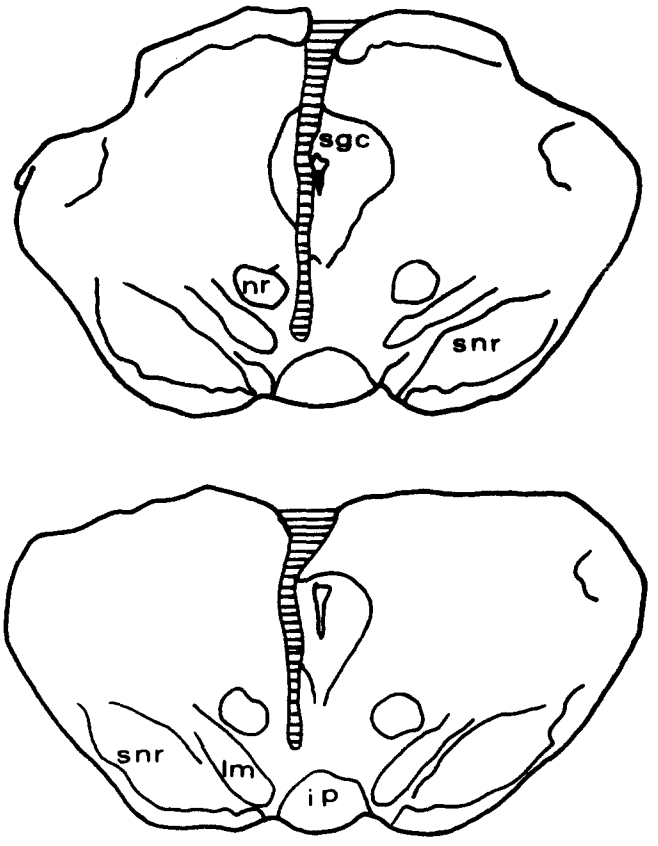

FIG. 1. Drawings of fresh brain sections of two rats showing electrode locations in the ventral tegmental area of the mesencephalon. The cortex is not represented. IP=nucleus interpedoncularis: lm=leminscus medialis: nr=nucleus ruber: snr=substantia nigra reticulata: sgc=substantia grisea centralis.

Exploration induced significant changes in the concentration of c-AMP and c-GMP in the striatum and cortex. The cortical changes may be related to the exploratory behavior. On the other hand, identical changes are seen in the striatum following either ICSS or open-field exploration. This strongly suggests that biochemical changes observed in the striatum may be related to the motor activity accompanying both experimental situations. As in the previous experiment, changes in c-AMP and c-GMP occur in opposite directions. The non-significant variations encountered in the hippocampus, following open-field activity, do not follow the aforementioned pattern and consequently could not be suspected to be of the same origin than those seen following ICSS.

An artifactual explanation (anoxia and post-mortem metabolism due to the *near freezing* technique) of changes observed in the present experiments seems unlikely since the same procedure should have affected the control as well as the experimental animals. Ipsilateral versus contralateral assays in the same animal did not seem appropriate in the present study since electrodes were located only 400 microns apart from the midline.

The striatal changes were not specific to the ICSS group and therefore could not be attributed to the rewarding proc-

TABLE 1

MEAN BRAIN VALUES ± STANDARD ERRORS OF C-AMP AND C-GMP IN CONTROL (C) AND SELF-STIMULATING RATS (SS). C-AMP AND C-GMP ARE EXPRESSED IN P MOLES/MG OF TISSUE AND FENTO MOLES/MG OF TISSUE RESPECTIVELY. P VALUES REPRESENT THE PROBABILITY THAT THE OBSERVED DIFFERENCES OCCUR RANDOMLY (T-STUDENT TEST)

		Olf bulbs	Cortex	Striatum	Hippocampus	Hypothalamus	Pons	Medulla
c-AMP	C	0.58 ± 0.041	0.55 ± 0.038	1.105 ± 0.084	0.78 ± 0.052	1.31 ± 0.12	0.51 ± 0.034	1.60 ± 0.124
	SS	0.60 ± 0.071	0.55 ± 0.072	0.850 ± 0.072	0.98 ± 0.064	1.39 ± 0.081	0.50 ± 0.022	1.62 ± 0.16
				0.02<p<0.05	0.02<p<0.05			
c-GMP	C	32.98 ± 2.99	38.98 ± 4.49	29.09 ± 3.75	24.75 ± 2.25	49.48 ± 2.99	32.98 ± 3.00	46.48 ± 5.98
	SS	34.48 ± 4.50	41.98 ± 2.99	44.38 ± 5.40	17.99 ± 0.75	44.98 ± 1.49	34.49 ± 1.49	43.48 ± 4.50
				0.02<p<0.05	0.02<p<0.05			

TABLE 2

MEAN BRAIN VALUES ± STANDARD ERRORS OF C-AMP AND C-GMP IN CONTROL (C) AND EXPLORATING RATS (E). C-AMP AND C-GMP ARE EXPRESSED IN P MOLES/MG OF TISSUE AND FENTO MOLES/MG OF TISSUE RESPECTIVELY. TESTS OF SIGNIFICANCE AS IN TABLE 1

		Olf bulbs	Cortex	Striatum	Hippocampus	Hypothalamus	Pons
c-AMP	C	0.93 ± 0.17	0.57 ± 0.08	1.04 ± 0.07	0.84 ± 0.12	1.32 ± 0.15	0.71 ± 0.05
	E	0.84 ± 0.12	1.00 ± 0.11	0.83 ± 0.03	0.75 ± 0.07	1.22 ± 0.11	0.65 ± 0.07
			p=0.02	p=0.02			
c-GMP	C	38.39 ± 3.34	39.03 ± 3.43	30.23 ± 2.94	21.26 ± 4.22	51.34 ± 3.31	31.10 ± 2.76
	E	37.13 ± 2.61	26.36 ± 3.82	49.12 ± 4.94	17.42 ± 2.58	51.74 ± 3.51	34.49 ± 1.29
			0.02<p<0.05	p<0.01			

ess. This observation is of interest in light of previous observation that ICSS in the VTA failed to increase the release of dopamine in the striatum [1, 15, 21, 27]. Furthermore, the present pattern of changes (decreased c-AMP and increased c-GMP) can also be produced by acetylcholine [2,11] and therefore may implicate the involvement of this neurotransmitter.

On the other hand, changes in the hippocampus were observed only in the ICSS group and are thus likely ascribed to the rewarding neuronal activity. There are, however, several reasons that can oppose the above contention:

(1) Motor activity in our control animals (open-field test) was somewhat different from the regular lever-pressing behavior exhibited in ICSS. Unfortunately, identical motor activity is difficult to achieve in control and self-stimulating rats. Furthermore, rewarding stimulation delivered by the experimenter in immobile rats is not feasible since the stimulation renders the animals hyperactive [14].

(2) The well established increase of theta activity in the hippocampus following ICSS [3, 8, 10] is believed to be dependent on the amplitude of the accompanying motor behavior rather than on the rewarding stimulation [17]. It should be noted, however, that a relationship between theta activity and biochemical events in the hippocampus has not yet been established.

(3) There is a large amount of data suggesting that hippocampus is involved in motor behavior, especially in voluntary phasic activity [32]. Consequently, biochemical changes in the hippocampus as seen in the present experiment may have resulted from the rat's phasic pressing activity rather than from contingent reinforcement.

(4) The observed changes may have been produced by irradiation of the current in non-rewarding hippocampus-afferent fibers near the electrode. Indeed, VTA is very heterogeneous and comprises, in addition to the A8, A9 and A10 dopaminergic cell groups [5], serotonergic [5] as well as acetylcholinergic fibers of passage [24].

On the other hand, the contention that c-AMP and c-GMP changes in the hippocampus are related to the rewarding neuronal activity may partly be supported by the following considerations:

(1) Electrode placements in the rat hippocampus sustain ICSS [30].

(2) ICSS is obtained in three major afferent structures to the hippocampus, i.e., septum [4,16], dorsal noradrenergic bundle [4], and median raphe [13, 20, 25, 31]. It was found by Segal and Bloom [22] that rewarding stimulation of the locus coeruleus results in drastic changes of hippocampal electrical activity. In addition, an increased concentration of norepinephrine in the hippocampus [27] and the LC-dorsal norepinephrinic bundle [15,21] was found following ICSS in the VTA. However, the β-adrenergic receptors do not seem to be involved in ICSS [33], suggesting that other hippocampal inputs (serotonergic and/or acetylcholinergic) may be responsible for the changes observed in the present study.

To our knowledge, the present work is the first that describes changes in brain concentration of c-AMP and c-GMP following ICSS. It was shown first, that spontaneous or electrically induced behaviors can result into measurable changes of the endogeneous concentration of these nucleotides and that these changes are region-specific. It is obvious that this finding may be of great potential usefulness in

the search of links between different behaviors and brain regions. Second, and in agreement with other investigators, the hippocampus seems to be involved in intracranial reward. However, a possible link between the observed nucleotide changes and known neurotransmitters was not permitted by the present results. Third, in all regions where significant changes were observed, c-AMP and c-GMP concentrations varied in opposite directions. This last observation cannot be explored in the present work but supports the contention that a functional relationship between c-AMP and c-GMP may exist.

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