VENTRAL HIPPOCAMPAL MEDIATOR MECHANISMS IN ANXIETY STATES FORMED BY VARIOUS AVERSIVE SITUATIONS

A. N. Talalaenko, G. K. Krivobok, Yu. V. Stakhovskii, A. V. Chernikov, A. A. Shekhovtsov, and S. L. Shevchenko

UDC 616.895.4-02:615.214.32]-07

KEY WORDS: hippocampus; anxiety; neurochemical mechanisms.

The hippocampus is not only one point of the focus of motivation formed during stress, but also an anatomo-physiological brain structure that belongs to the system controlling anxiety states of varied aversive genesis [2, 3]. The functional role of the mediator systems of this formation of the archipaleocortex still remains unexplained. The anxiolytic effect of diazepam, revealed by various tests of anxiety [7], correlates in radioligand studies with its specific binding by membranes of hippocampal neurons, leading to increased affinity of $GABA_A$ receptors for the corresponding mediator [5]. Meanwhile application of several serotonin agonists (buspirone and insapirone, which simulate its effects) in the hippocampus clearly potentiates the effects of aversive brain stimulation under conditions of punishable behavior [9]. However, besides GABA and serotinin, the hippocampus also has a dopaminergic and glutamatergic synaptic inflow responsible for the emotional state [6, 12], but its contribution to the realization of anxiety has not yet been studied.

In view of the facts described above it was decided to inject GABA, serotonin (5-HT), dopamine (DA), and L-glutamic acid (GA) directly into the ventral hippocampus (VH) in order to evaluate their functional role and to discover their role in the genesis of anxiety states formed by aversive situations affecting different modalities.

EXPERIMENTAL METHOD

Experiments were carried out on 15 noninbred mature male rats weighing 290 ± 30 g, in which an anxiety state was simulated by the method of avoiding an illuminated area and a threatening situation, as described previously [4]. After implantation of chemical electrodes under ether anesthesia, taking stereotaxic coordinates from [1], into VH (AP 5.0, L 3.5, H 6.8), 1 μl of a solution of GABA, DA, GA (10 μg) and 5-HT (serotonin-creatinine sulfate, 20 μg) was injected by means of a microinjection system into the trained animals. After 5 min the rats were placed in the experimental situation in which, just as previously [4], changes in parameters of motivated behavior, indicating the presence of an anxiolytic action, were determined by means of a decatron-based recording system.

In the control experiments, 1 μl of 0.9% NaCl solution was applied to VH. The results were subjected to statistical analysis in the usual way. After the end of the experiments the rats were killed under ether anesthesia. To verify the position of the chemical electrodes, the animals' brain was investigated morphologically.

Department of Pharmacology, M. Gor'kii Donetsk State Medical Institute. (Presented by Academician of the Russian Academy of Medical Sciences K. V. Sudakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 10, pp. 374-376, October, 1992. Original article submitted November 15, 1991.

 0007-4888/92/0010-1450$12.50

TABLE 1. Effect of Monoamines and Amino Acids Injected into Ventral Hippocampus on Anxiety States in Tests of Avoidance of an Illuminated Area (numerator) and a Threatening Situation (denominator; M ± m, n = 5)

Substance	Dose, μg	Time spent by rats in illuminated compartment, sec	Motor activity, number of squares crossed in floor in illuminated compartment	Intensity of motivation for rats to remain in dark compartment, conven.units
NaCl (0.9% solution)	1 μliter	3,0±0,45 / 2,2±0,37	5,6±0,24 / 5,2±0,45	0,59±0,06 / 0,61±0,06
Dopamine	10	3,6±0,51 / 4,0±0,44*	6,0±0,32 / 5,8±0,20	0,54±0,08 / 0,44±0,03*
Serotonin	20	3,4±0,51 / 4,6±0,62*	5,8±0,37 / 6,2±0,20	0,54±0,07 / 0,40±0,04*
GABA	10	5,8±0,58* / 2,4±0,56	6,4±0,25* / 5,6±0,25	0,42±0,03* / 0,58±0,04
L-glutamic acid	10	3,8±0,73 / 2,8±0,58	6,0±0,32 / 5,8±0,49	0,58±0,06 / 0,54±0,09

Legend. *$p \leq 0.05$ – Values differing significantly from control.

EXPERIMENTAL RESULTS

The morphologic investigations showed that DA, 5-HT, GABA, and GA acted on neurons of VH. Local applications of mediators to this brain formation revealed the different roles of certain neurochemical systems of VH in anxiety states of different aversive genesis.

It will be clear from Table 1 that the anxiety state formed by the motivation of fear [4] was resistant to chemical stimulation of VH by DA, 5-HT, and GA, which had no significant effect on the parameters of avoidance of the illuminated area. Conversely, microinjection of GABA into VH caused a distinct antiaversive effect, manifested as an increase in the length of time spent by the rats in the illuminated compartment and reduction of the intensity of their motivation for staying in the dark compartment. The antianxiety action thus revealed did not correlate with a motor deficiency of skill performance, for when GABA was injected locally into VH, it stimulated the animals' motor activity. The facts described above are evidence that during the formation of a fear motivation the neuronal matrix of anxiety activates not the DA-ergic, 5-HT-ergic, and glutamatergic components, but the GABA-ergic mediator component of VH, which is functionally meaningful in negative emotional states. This conclusion is in agreement with those obtained with other approach techniques, indicating that GABA mechanisms are punishment systems, involved in the regulation of anxiety, and targets for the anxiolytic action of tranquilizers [5, 7].

Under free choice conditions between the dark and illuminated chambers chemical stimulation of VH of the rats by GABA and GA does not affect, whereas stimulation by DA and 5-HT counteracts the anxiety state formed by aversive action of different biological significance [4] (the avoidance reaction of the spectator rats against the background of nociceptive stimulation of the victim rats). This anxiolytic effect is selective, for in the test of avoidance of a threatening situation DA and 5-HT by reducing the intensity of the motivation of finding rats in the dark compartment and increasing their length of stay in the lit compartment, do not affect the animals' motor activity (Table 1). It can be concluded from these findings that the neuronal matrix of anxiety, formed on the basis of negative zoosocial emotional-stress reactions activates, not glutamatergic or GABA-ergic, but DA-ergic and 5-HT-ergic mediator components of VH. This conclusion is satisfactorily confirmed by investigations showing the ability of stress-induced aversive influences of different modalities to exert a significantly different influence on the hippocampal 5-HT level [10, 11] and it is in accordance with information showing that different neurochemical activators of dopaminergic systems are characterized by opposite effects [8].

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