

BEHAVIORAL AND NEUROCHEMICAL ALTERATIONS FOLLOWING 5,6-DIHYDROXYTRYPTAMINE ADMINISTRATION INTO THE MEDIAN RAPHE NUCLEUS IN THE CAT

Andrzej ROMANIUK, Milena KOPROWSKA, Magdalena STRZELCZUK and
Marek WIECZOREK

Department of Neurophysiology, Institute of Physiology and Cytology,
University of Łódź, 66 Rewolucji 1905 r. St., 90-222 Łódź, Poland

Key words: intrahypothalamic carbachol, defensive behavior, median raphe nucleus lesion, brain monoamines, cat

Abstract. The 5,6-dihydroxytryptamine administration into the median raphe nucleus in the cat resulted in an increase in the carbachol-induced emotional-defensive behavior and a decrease of 5-hydroxyindoleacetic acid and dopamine level in the hypothalamus, midbrain and amygdala. Lesions of median raphe nucleus resulted in similar, but more subtle alterations of post-carbachol emotional-defensive response similarly to dorsal raphe nucleus lesions, and they had also different biochemical effects in the "emotional regions" of the brain (hypothalamus, midbrain, amygdala). The absence of differentiated participation of both dorsal raphe nucleus and median raphe nucleus in the regulation of different aspect of the cat's behavior, and the role of both nuclei in the modulation of post-carbachol response is discussed.

INTRODUCTION

It is well documented that the major part of ascending serotonergic neurons in the midbrain dorsal raphe nucleus (DRN) and the midbrain median raphe nucleus (MRN) form two neuronal systems, the mesocortical and mesolimbic, which project to different forebrain structures (1, 4, 12). The fact that there are anatomical differences in the projections from the DRN and MRN suggests that different raphe nuclei are in-

involved in the control of different aspects of behavior. As a rule various effects were obtained (5, 7-9, 11, 13, 20, 21) after the destructions of the DRN and MRN in experiments on rats. Fundamental differences consist in the fact that the destruction of the MRN significantly potentiates locomotor activity whereas lesions of the DRN are without effect (5, 7-9). Lesions of the DRN produce muricide in non-killing rats and increase in pain elicited aggression, whereas lesions to the MRN do not produce aggressive behavior (8, 21). Thus, it has been demonstrated that lesions to the DRN and MRN result in a similar fall in the level of serotonin (5-HT) in the cerebral cortex, hypothalamus and striatum, however, different in the hippocampus. The DRN lesion produced a minimal, non-significant (10%) reduction in the hippocampal 5-HT level, while the MRN lesion caused an 80% reduction (5, 8, 9, 11, 18). All the data obtained indicate that only the DRN participates in the regulation of aggressive behavior.

In our previous investigation (15) we found that 5,6-dihydroxytryptamine (5,6-DHT) administration to the DRN of the cat resulted in an increase in the carbachol-induced emotional defensive behavior, a decrease in the concentration of noradrenaline (NA), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the "emotional regions" (hypothalamus, midbrain, amygdala) of the brain, and an increase in the hypothalamic dopamine (DA) level. In continuation of this research, the present study examined the behavioral and neurochemical effects of chemical lesions of the MRN with respect to carbachol-induced emotional-defensive behavior.

MATERIAL AND METHODS

Subjects and surgery

The experiments were carried out on 18 cats of both sexes. All cats had two cannulas chronically bilaterally implanted in the antero-medial hypothalamus according to the stereotaxic coordinates of Snider and Nemer's atlas (19): A = 13.0, L = 1.5, H = -3.0 and one cannula implanted in the MRN: P = 2.0, L = 0.0, H = -4.5. The cannula was inserted at an angle of 33° after appropriate trigonometric calculations. The details of the surgery and microinjection procedure were described earlier (16).

Drugs

Carbachol (carbachol puriss, Koch-Light) was dissolved in a 0.9% NaCl solution at 20°C and injected bilaterally 4 µg/1 µl into each part

of the hypothalamus. 5,6-DHT (5,6-dihydroxytryptamine creatinine sulfate, Sigma) was dissolved immediately before use in a 0.2% solution of ascorbic acid (AA) in saline at 4°C and injected into the MRN — 25 µg/2 µl. A control group received 2 µl AA into the MRN.

Experimental procedure

The intensity of the emotional-defensive behavior evoked by intrahypothalamic carbachol injections was evaluated by recording the latency period of growling response (L), the total number of growls (N), the total time of their duration (T) and the total time duration of vocalization (D) (for details see 3, 16). The response was considered completed if a growl was not followed by another within 3 min. All cats were tested twice at 7 day intervals for growling response, and mean values from these two experiments established the control level for the data obtained 5, 10, and 15 days after 5,6-DHT or AA injection into the MRN. The animals were then divided into two groups:

Group 1 ($n = 6$). The cats were injected into the MRN with 0.2% AA in a volume of 2 µl, and intensity of carbachol-induced growling response was tested 5, 10 and 15 days after injection.

Group 2 ($n = 12$) like Group 1, but 5,6-DHT in a dose of 25 µg/2 µl was injected into the MRN.

All the cats were killed by decapitation 24 h after the last test for carbachol-induced growling; their brains were rapidly removed and four structures, i.e. the anterior hypothalamus (HA), the posterior hypothalamus (HP), the midbrain central grey matter (GC) and the amygdala (AM) were separated with dissection and kept frozen (for details see 17). Concentrations of NA, DA, 5-HT and 5-HIAA in HA, HP, GC and AM were determined spectrofluorometrically according to the method of Earley and Leonard (6).

Statistics

Results were analyzed by ANOVA (mixed design) followed by Duncan's multiple comparison test.

RESULTS

Biochemical data

Regional brain concentrations of NA, DA, 5-HT and 5-HIAA in Group 1 (control) and Group 2 (MRN lesioned) are presented in Table 1. ANOVA demonstrated significant differences between the groups in the contents of DA and 5-HIAA, which were $F_{1/16} = 15.09$, $p < 0.002$ and

TABLE 1

Regional brain concentrations of NA, DA, 5-HT and 5-HIAA 16 days after 5,6-DHT injections to the median raphe nucleus

Group	Brain region	Amine content in $\mu\text{g/g}$ wet tissue (mean \pm SEM)			
		NA	DA	5-HT	5-HIAA
1. Control	HA	1.497 \pm 0.234	4.246 \pm 0.787	1.292 \pm 0.328	0.611 \pm 0.106
2. Lesioned		1.108 \pm 0.158	1.719 \pm 0.429 $p < 0.01$	0.760 \pm 0.101	0.374 \pm 0.041 $p < 0.01$
1. Control	HP	1.020 \pm 0.257	2.404 \pm 0.313	1.049 \pm 0.282	0.741 \pm 0.095
2. Lesioned		0.725 \pm 0.089	1.317 \pm 0.186 $p < 0.01$	0.916 \pm 0.082	0.394 \pm 0.025 $p < 0.01$
1. Control	GC	0.613 \pm 0.153	2.136 \pm 0.311	1.189 \pm 0.275	0.790 \pm 0.130
2. Lesioned		0.389 \pm 0.044	1.262 \pm 0.116 $p < 0.01$	1.119 \pm 0.091	0.444 \pm 0.24 $p < 0.01$
1. Control	AM	0.545 \pm 0.180	4.785 \pm 0.785	0.698 \pm 0.192	0.601 \pm 0.080
2. Lesioned		0.397 \pm 0.102	2.965 \pm 0.402 $p < 0.01$	0.640 \pm 0.086	0.439 \pm 0.072 $p < 0.01$

Statistical significance: Duncan's test

$F_{1/16} = 13.95$, $p < 0.002$ respectively. Duncan's test showed that the level of DA and 5-HIAA was lower in Group 2 than in Group 1 ($p < 0.001$). Moreover, ANOVA demonstrated that there were significant differences between particular brain regions for NA ($F_{3/48} = 25.92$, $p < 0.001$), for DA ($F_{3/48} = 14.92$, $p < 0.001$) and for 5-HT ($F_{3/48} = 10.19$, $p < 0.001$). There were no differences in the contents of 5-HIAA. Only with respect to 5-HIAA there occurred a significant interaction between brain regions and groups ($F_{3/48} = 2.87$, $p < 0.045$).

Post-carbachol growling response

Post-carbachol growling response parameters, i.e. latent period (L), number of growls (N), time of their duration (T) and time duration of vocalization response (D) are presented in Fig. 1. ANOVA demonstrated that there were no differences between time period (growling response before and 5, 10 and 15 days after AA or 5,6-DHT treatment) as well as between the groups (control and lesioned) for L and N. Nonetheless, significant differences occurred in time period for T ($F_{3/48} = 7.29$, $p < 0.001$) and for D ($F_{3/48} = 6.67$, $p < 0.001$). There were no significant differences between the groups, but interactions between time periods and groups were significant for T ($F_{3/48} = 2.93$, $p < 0.05$) and for D ($F_{3/48} = 3.45$, $p < 0.023$). Duncan's test for interactions showed a significant increase in T and D in Group 2, detailed differences being the following:

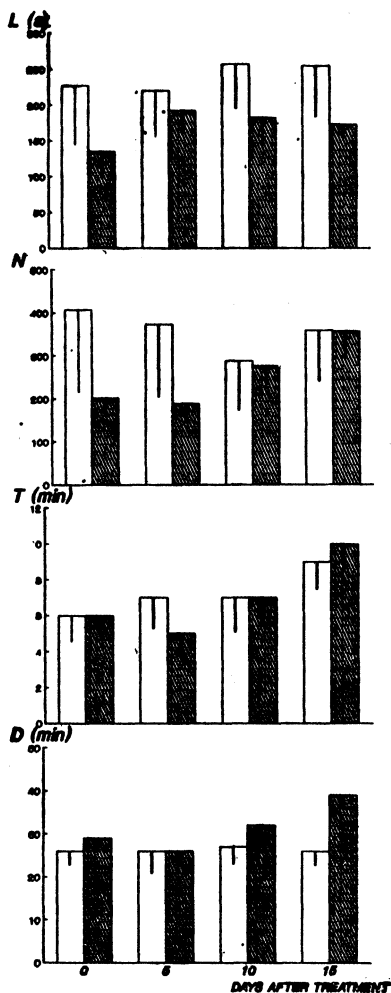


Fig. 1. The effect of ascorbic acid (white bars) and 5,6-dihydroxytryptamine (dashed bars) administration to the median raphe nucleus on growling response evoked by intrahypothalamic carbachol injections. Mean latent period of growling response (L), mean number of growls (n), mean time of their duration (T) and mean time duration of vocalization (D) \pm SEM. O, control level before treatment.

for T — after 15 days vs. control ($p < 0.01$), 15 days vs. 5 days ($p < 0.001$), 15 days vs. 10 days ($p < 0.05$) and for D — 15 days vs. control ($p < 0.001$), 15 days vs. 5 days ($p < 0.001$), 15 days vs. 10 days ($p < 0.01$) and 10 days vs. 5 days ($p < 0.05$).

DISCUSSION

Following 5,6-DHT injection into the MRN, an increase in post-carbachol growling response was observed, which speaks for a rise in the central emotional-defensive arousal (3). This behavioral alteration was accompanied by neurochemical changes, as evidenced by a drop in the 5-HIAA level in HA, HP, GC and AM as well as a drop in the level of

DA in the same structures; this speaks for the attenuating of the serotonergic system activity and the lowering of the DA concentration in the emotional structures of the brain. An increase in post-carbachol growling response was similar, but more subtle than that following the injection of the same neurotoxin into the DRN as in the preceding investigation (15). After the MRN lesions, like after the DRN lesions previously no visible other behavioral changes were noticed. Thus, it appears that in the cat there is no functional differentiation of the MRN and DRN and both nuclei participate in the regulation of emotional-defensive behavior, their function being inhibitory. The result is unexpected because numerous experiments carried out on rats showed that the MRN and DRN — are functionally differentiated and only DRN participates in the regulation of defensive behavior (see review 14). What therefore are the reasons of divergences occurring in the results obtained in the investigation on rats and in our experiments carried out on cats? They can hardly be related to differences between the species. Though cats are very different from rats as far as their behavior is concerned, in the literature there is no explicit and convincing evidence of dissimilarity between these two species as regards neuronal and neurochemical regulation of emotional-defensive behavior. However, one may suppose that those differences result from two reasons: (1) a dissimilarity in models of emotional-defensive responses. Experiments carried out on rats use emotional-defensive responses induced by pain or fear with the participation of the whole group of exteroceptive stimuli and the responses are of offensive character (aggression). In our model there are no nociceptive stimuli at all, and exteroceptive stimuli at the initial stage are of no significance because the reaction initiation occurs by a direct excitation of the hypothalamic motivational centres and the response itself has the nature of a threat so it is an offensive-defensive one. (2) Similar alterations in post-carbachol growling response after the MRN and DRN lesions might as well result from the fact that the hypothalamus, from which the post-carbachol response was evoked, is innervated by both nuclei in the same proportion (12). Thus, it can be assumed that as a result of 5-HT neurons damage in the MRN and DRN a similar attenuation of inhibitory influence of the serotonergic system in the area of hypothalamus occurs and therefore in both cases a similar rise in post-carbachol emotional-defensive response is noticed.

A question also arises why after the MRN lesions an increase in post-carbachol response was expressed only in an increase of T and D while other parameters (L and N) did not undergo significant changes. Latency is not a direct indicator of post-carbachol defensive response because it depends, above all, on the diffusion rate of intrahypothala-

mically administered carbachol, which in turn, depends on the neuronal elements where the tip of injection cannula is placed and on the degree of necrotic changes in the injection area. This parameter measurements may, however, constitute an indirect indication as regards possible functional alterations of hypothalamic cholinergic receptors which may occur following some particular lesions of the brain. A lack of significant changes of N while T and D increase at the same time after the MRN lesions may suggest that there occurs a quantitative change in the post-carbachol defensive response, which consists in the fact that the intensification of growling response occurs by extension of a particular growl duration and consequently leads to a longer period of vocalization response. Moreover, one may suppose that relatively subtle quantitative changes of some parameters of the post-carbachol growling response indicate that the MRN plays a role of lesser importance in the emotional-defensive regulation than the DRN. These slight alterations may result from the fact that in the cat's MRN only 35% of 5-HT neurons have been discovered while in the DRN — 70% (22).

After the MRN lesions not only an attenuation of activity of the serotonergic system was observed, but also a significant drop in the DA level. Recently, it was demonstrated by means of histochemical and immunochemical methods that in the nuclei raphe there are also non-serotonergic cells which contain other neurotransmitters and neuromodulators (2). At the same time, it is known that all neurotoxins which selectively damage particular kinds of neurons have only a relatively selective action (10). Thus, it can be assumed that a drop in DA in all the investigated brain structures results from a damage of dopaminergic neurons within the MRN and above all, from a damage of dopaminergic neurons of group A10 located within the ventral tegmental area close to the MRN.

This investigation was supported by Project CPBP 04.01. of the Polish Academy of Sciences.

REFERENCES

1. ANDEN, N. E., DAHLSTRÖM, A., FUXE, K., LARSSON, K., OLSON, L. and UNGERSTEDT, U. 1966. Ascending monoamine neurons to the telencephalon and diencephalon. *Acta Physiol. Scand.* 67: 313 - 326.
2. BOWKER, R. M., WESTLUND, K. N., SULLIVAN, M. C., WILBER, J. F. and COULTER, J. D. 1983. Descending serotonergic, peptidergic and cholinergic pathways from the raphe nuclei: a multiple transmitter complex. *Brain Res.* 288: 33 - 48.
3. BRUDZYŃSKI, S. M. 1981. Growling component of vocalization as a quantita-

- tive index of carbachol-induced emotional-defensive response in cats. *Acta Neurobiol. Exp.* 41: 33 - 51.
4. DAHLSTRÖM, A. and FUXE, K. 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system: I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand. (Suppl. 232)*, 62: 1 - 55.
 5. DRAY, A., DAVIES, J., OAKLEY, N. R., TONGROACH, P. and VELLUCCI, S. 1978. The dorsal and median raphe projections to the substantia nigra in rat: electrophysiological, biochemical and behavioral observations. *Brain Res.* 151: 431 - 442.
 6. EARLEY, C. J. and LEONARD, B. E. 1978. Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine, and several metabolites from tissue using disposable bio-rad columns packed with Sephadex G-10. *J. Pharmacol. Methods* 1: 67 - 79.
 7. GELER, M. A., PUERTO, D. B., MENKES, D. B., SEGAL, D. S. and MANDELL, A. J. 1976. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. *Brains Res.* 106: 257 - 270.
 8. JACOBS, B. L. and COHEN, A. 1976. Differential behavioral effects of lesions of the median or dorsal raphe nuclei in rats: open field and pain-elicited aggression. *J. Comp. Physiol. Psychol.* 90: 102 - 108.
 9. JACOBS, B. L., WISE, W. D. and TAYLOR, K. M. 1974. Differential behavioral and neurochemical effects following lesions of the dorsal or median raphe nuclei in rats. *Brain Res.* 79: 353 - 361.
 10. JONSSON, G. 1983. Chemical lesioning techniques: Monoamine neurotoxins. In A. Björklund and T. Hökfelt (ed.), *Handbook of chemical neuroanatomy*. Vol. 1. *Methods in chemical neuroanatomy*. Elsevier, Amsterdam, p. 463 - 507.
 11. LORENS, S. A. and GULDBERG, H. C. 1974. Regional 5-hydroxytryptamine following selective midbrain raphe lesions in the rat. *Brain Res.* 78: 45 - 56.
 12. NIEUWENHUYS, R. 1985. *Chemoarchitecture of the brain*. Springer-Verlag, Berlin, 246 p.
 13. PRZEWŁOCKA, B., STALA, L., SCHEL-KRÜGER, J. and PRZEWŁOCKI, R. 1986. Different behavioral responses of rats to kainate injections into dorsal and median raphe nuclei. *Pol. J. Pharmacol. Pharm.* 38: 509 - 519.
 14. PUCIŁOWSKI, O. and KOSTOWSKI, W. 1983. Aggressive behaviour and the central serotonergic systems. *Behav. Brain Res.* 9: 33 - 48.
 15. ROMANIUK, A., FILIPCZAK, M. and FRYCZAK, J. 1987. The influence of injection of 5,6-dihydroxytryptamine to the dorsal raphe nucleus on carbachol-induced defensive behavior and regional brain amine content in the cat. *Pol. J. Pharmacol. Pharm.* 39: 17 - 25.
 16. ROMANIUK, A. and GOŁĘBIEWSKI, H. 1977. Midbrain interaction with the hypothalamus in expression of aggressive behavior in cats. *Acta Neurobiol. Exp.* 37: 83 - 97.
 17. ROMANIUK, A., STRZELCZUK, M. and WIECZOREK, M. 1989. Serotonin depletion with p-chlorophenylalanine in the cat: effects on carbachol-induced defensive behavior and regional brain amine content. *Acta Neurobiol. Exp.* 49: 130 - 140.
 18. ROMMELSPACHER, H. and STRAUSS, S. 1980. Effect of lesions of raphe nu-

- clei on the activity of catecholaminergic and serotonergic neurones in various brain regions of the rat in vivo. *J. Neural Transm.* 49: 51 - 62.
19. SNIDER, R. J. and NIEMER, W. T. 1961. A stereotaxic atlas of the cat brain. Univ. Chicago Press, Chicago.
 20. SREBRO, B. and LORENS, S. A. 1975. Behavioral effects of selective raphe lesions in the rat. *Brain Res.* 89: 303 - 325.
 21. WALDBILLING, R. J. 1979. The role of the dorsal and median raphe in the inhibition of muricide. *Brain Res.* 160: 341 - 346.
 22. WILKLUND, L., LÉGER, L. and PERSSON, M. 1980. Monoamine cell distribution in the cat brain stem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups. *J. Comp. Neurol.* 203: 613 - 647.

Accepted 18 May 1990