Pharmacology Biochemistry & Behavior, Vol. 28, pp. 101–103. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

The Involvement of Neuropeptide Y in the Antimuricide Action of Noradrenaline Injected Into the Medial Amygdala of Olfactory Bulbectomized Rats

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Received 1 January 1987

KATAOKA, Y., Y. SAKURAI, K. MINE, K. YAMASHITA, M. FUJIWARA, M. NIWA AND S. UEKI. *The involvement of neuropeptide Y in the antimuricide action of noradrenaline injected into the medial amygdala of olfactory bulbectomized rats.* PHARAMACOL BIOCHEM BEHAV **28**(1) 101–103, 1987.—The present study was designed to clarify the functional role of neuropeptide Y (NPY) in the regulation of muricide induced by olfactory bulbectomy (OB) in relation to that of noradrenaline (NA) in the medial amygdala (AME). NA injected into AME inhibited muricide dose-dependently in OB rats. NPY at doses of 5 and 10 μg/μl injected alone into AME failed to suppress muricide. When NPY 10 μg was injected into AME in combination with the maximal non-effective dose of NA, which was determined in each rat, muricide was suppressed in 80% of OB rats. The present study has provided the first evidence suggesting that NPY may be involved in the regulation of OB-induced muricide.

Neuropeptide Y Noradrenaline Muricide Olfactory bulbectomy Rat

OLFACTORY bulbectomy (OB)-induced muricide (mouse-killing behavior) has been known to be a useful laboratory model for evaluating the property of antidepressants [21]. Studies with brain lesioning and microinjection of drugs have suggested that the lateral hypothalamus (LH) and medial amygdala (AME) play important roles in the regulation of muricide [8, 18, 22]. In addition, destruction and stimulation of the locus coeruleus facilitated and inhibited the occurrence of muricide in OB rats, respectively [17,23]. These results suggest that noradrenergic mechanism may play an inhibitory role in muricide. This view was supported by our recent findings that the content of noradrenaline (NA) in LH, ventromedial hypothalamus and AME increased and the turnover rate of NA in these brain regions decreased in OB rats [13,14].

On the other hand, neuropeptide Y (NPY) is a 36-amino acid residue peptide first isolated from porcine brain by Tatemoto *et al.* [20]. NPY was found to have a wide spread, characteristic distribution in rat and human brain [1, 2, 4]. Especially, high concentration of NPY immunoreactivity was demonstrated in many regions of the hypothalamus and AME [4] which has been known to regulate emotional behavior. NPY immunoreactivity has been shown to co-exist within NA-ergic and adrenergic neurons [5, 11, 12]. It is of interest that a functional interaction between catecholamines and NPY has been suggested by experiments in the peripheral tissues [3,16]. However, the functional role of NPY in the central nervous system remains unknown.

The present study was therefore designed to clarify the functional role of NPY in the regulation of muricide induced by OB in relation to that of NA in AME.

METHOD

Male Wistar King A rats supplied by Seiwa Experimental Animal Ltd., aged 8 weeks and weighing 180–200 g at the beginning of the experiment were used. The animals were housed in groups of 5 animals each, maintained on 23±2°C with light-dark cycle (lights on 7:00 a.m.) and given food and water ad lib. Before brain surgery, the animals were trans-

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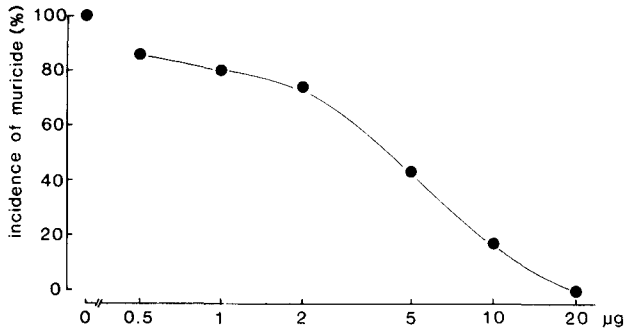

FIG. 1. Dose-response curve for antimuricide action of noradrenaline injected into the medial amygdala of olfactory bulbectomized rats. Doses of drugs administered were expressed as μg weight (base)/μl/side × 2.

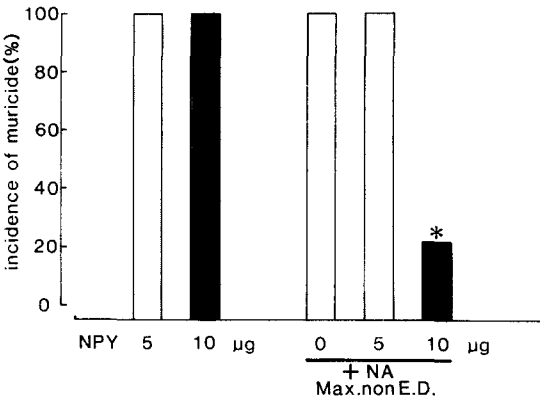

FIG. 2. Effect of neuropeptide Y (NPY) and NPY combined with maximal non-effective dose (Max. non E.D.) of noradrenaline (NA) injected into the medial amygdala on muricide in olfactory bulbectomized rats. Max. non E.D. of NA was determined for each rat among 0.5, 1, 2, 5 and 10 μg. *$p<0.05$; significantly different from NPY 10 μg alone (Fisher exact probability test, one-tailed). Doses of drugs administered were expressed as μg weight (base)/μl/side × 2.

ferred to the individual cages. Muricide test was performed by putting white male mouse in the cage after the individual housing for 1 hr. Only rats which did not show muricide within 30 min after introducing a mouse in the cage were subjected to brain lesioning. The olfactory bulbs of these animals were bilaterally removed by suctioning under pentobarbital anesthesia (40 mg/kg IP). The rats showing muricide within 3 min in the test 2 weeks after OB were subjected to the surgery for the guide cannula implantation. The guide cannula (0.7 mm in outer diameter) were bilaterally implanted into the AME under pentobarbital anesthesia according to the atlas of König and Klippel [15] (5.7 mm anterior from the lambda, 3.5 mm lateral to the midline and 7.9 mm horizontal from the skull). The microinjection was started 14 days after the cannula implantation.

NPY (Peninsula) and NA hydrochloride (Sigma) were dissolved in 10 μM citric acid containing 0.1% bovine serum albumin and distilled water, respectively. One μl of drug was bilaterally injected into AME. Doses of drugs administered were expressed as μg weight (base)/μl/side × 2. An injection cannula extending 1.0 mm below the tip of the guide cannula was used for microinjection of drugs. Injection rate was 1 μl/3 min, with the cannula left in place for additional one minute. The interval between injections was more than 3 days. NPY was injected only once into the same animal.

At 5, 10, 15 and 30 min after the microinjection of drugs into AME, muricide was tested for 3 min. When rats did not show muricide within 15 min after the injection, antimuricide action of drug was assessed as positive. When the antimuricide action of drug did not disappear within 30 min after injection, the muricide test was further repeated at an interval of 30 min until the rat recovered muricide. Only rats in which muricide was not inhibited by vehicle but inhibited by 20 μg of NA injected into AME were supplied for the drug experiment.

After the experiment was terminated, localization of the cannula tips was verified histologically. The rats in which both cannula tips were located out of AME were excluded from the experimental data.

RESULTS

NA injected into AME inhibited muricide dose-dependently in 30 OB rats (Fig. 1). The maximal non-effective dose (Max. non E.D.) of NA was determined among 0.5, 1, 2, 5 and 10 μg for each rat. Max. non E.D. of NA was 2 or 5 μg in 70% of OB rats used. As shown in Fig. 2, NPY alone at doses of 5 and 10 μg did not inhibit muricide in 5 OB rats each. Doses larger than 10 μg of NPY were not challenged in the present experiment. Then 5 or 10 μg of NPY combined with Max. non E.D. of NA were injected into AME in 6 and 9 OB rats, respectively. NPY at 10 μg combined with Max. non E.D. of NA, which alone failed to suppress muricide, significantly inhibited muricide in 7 out of 9 OB rats. After this experiment, Max. non E.D. of NA alone injected into AME did not inhibit muricide in these OB rats.

DISCUSSION

The present study suggests that NPY may facilitate the anti-muricide action of NA in AME. In the peripheral nervous system, NPY seems to act in the same direction as the co-existing transmitter NA to produce a vasoconstriction [16]. The view that the co-existing peptide (NPY) may support the action of the main transmitter, catecholamines, was pointed out in the central nervous system as well by the finding concerning with the hypotensive action of NPY [6]. On the other hand, hypothalamic NPY appears to stimulate feeding behavior independently of NA, although NPY and NA may still interact at the receptor level [7,19]. NPY has also been known to localize in the neurons containing somatostatin or GABA [9,10]. We cannot, therefore, exclude the possibility that NPY facilitates the antimuricide action of NA through the mechanisms other than NA-ergic mechanism.

In conclusion, the present study is the first demonstration for the involvement of NPY in the neuronal mechanism regulating OB-induced muricide.

ACKNOWLEDGEMENTS

This investigation was supported by the Grant-in-Aid for Scientific Research and the Grant-in-Aid for Encouragement of Young Scientists from the Japanese Ministry of Education, Science and Culture.

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