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A Genotype-Dependent Hippocampal Dynorphinergic Mechanism Controls Mouse Exploration

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VAN DAAL, J. H. H. M., Y. J. M. DE KOK, B. G. JENKS, S. E. WENDELAAR BONGA AND J. H. F. VAN ABEELEN. *A genotype-dependent hippocampal dynorphinergic mechanism controls mouse exploration.* PHARMACOL BIOCHEM BEHAV **28**(4)465–468, 1987.—Following microinjections with two dilutions of anti-dynorphin B antiserum into the hippocampal CA3 region, adult male mice from the inbred strains DBA/2 and C57BL/6 were individually tested for various exploratory behaviors in a novel environment and compared to preimmune serum control animals. Treatment augmented vertically-oriented exploratory acts in strain DBA/2 and reduced the scores in strain C57BL/6 so that strain differences originally present between the controls were reversed or eliminated after antiserum. These opposite effects indicate that a hippocampal dynorphinergic mechanism is involved in the regulation of novelty-induced behavior in mice and that its modulatory function depends on the genotype. It is concluded that DBA/2 animals exposed to novelty, as compared to C57BL/6, are characterized by an over-release of hippocampal dynorphin B which is neutralized in part by small amounts of antibody.

Hippocampus Dynorphin B Antiserum Opioid Peptide Exploratory behavior Novelty Genotype Inbred strains Mouse

HIPPOCAMPAL opioid peptides participate in the regulation of exploratory behavior in rodents [11]. There is evidence that exposure of mice to environmental novelty induces release of opioids [10] and there are strong indications that such neuropeptides act to disinhibit cholinergically-innervated pyramidal neurons in the CA1 and CA3 regions of the hippocampus by blocking inhibitory GABAergic interneurons [2,4]. The functioning of these neurotransmitter systems depends on the genotype, as appears from the opposite effects of intrahippocampal drug administration upon the exploration rates of mice from the inbred strains DBA/2 and C57BL/6 [8,10]. The opiate antagonist naloxone, for example, enhances exploration in the normally low-scoring DBA/2 and reduces it in the normally high-scoring C57BL/6, leading to a reversal of the original strain difference [8]. The agonist morphine decreases this behavior in both strains [8]. These observations suggest that the regulatory mechanism concerned operates efficiently in C57BL/6 animals: treatment with either agonist or antagonist will upset its optimally balanced peptidergic action, thereby depressing exploration. With regard to DBA/2 mice exposed to novelty, however, the above findings point to a less efficient mechanism, implying an overshoot in the release of one or more hippocampal opioid peptides which is, naturally, not alleviated by treatment with an agonist but can be attenuated by an appropriate inactivating agent: the latter will thus augment exploratory behavior.

One of the opioids present in the hippocampus is dynorphin B. As demonstrated by others in rats [1, 3, 5, 6, 12] and by us in mice (unpublished), it is confined to the mossy fibers which project onto the CA3 area. To ascertain the involvement of this neuropeptide in the genotype-dependent control of exploration, we injected a highly specific anti-dynorphin B antiserum into the dorsal hippocampus of DBA/2 and C57BL/6 mice, which were then tested in a novel environment. This attempt to partly inactivate released neuropeptide was expected to produce opposite effects on exploration rates in the two mouse strains. Our purpose was to establish whether an over-release of hippocampal dynorphin B in DBA/2 mice can be corrected by this treatment so that a more efficient disinhibition of exploratory responses to novelty becomes possible.

METHOD

Behavioral Testing

Laboratory conditions and behavioral recording methods were as described elsewhere [9,10]. Male mice, aged 13±1 weeks, from the strains DBA/2JNmg and C57BL/6JNmg [7] were placed individually for 20 minutes into a novel environment, an illuminated observation cage (108×49×49 cm), the floor of which was divided into 21 rectangles by painted lines and which contained a metal object attached 5 cm above the floor. The frequencies of the following behavioral acts were recorded: rearing (standing upright), incipient rearing, leaning against the wall, object-leaning, object-

TABLE 1

EXPLORATORY ACTS OBSERVED IN DBA/2 AND C57BL/6 MICE INJECTED INTRAHIPPOCAMPALLY WITH CONTROL SERUM AND WITH TWO DILUTIONS OF ANTI-DYNORPHIN B ANTISERUM: DATA OBTAINED AFTER WELL-AIMED INJECTIONS

	Control	1/226	1/113
Rearing			
C57BL/6	51.3 ± 5.1	29.2 ± 4.7‡	31.0 ± 4.1†
DBA/2	23.5 ± 3.1	43.1 ± 5.1‡	44.9 ± 4.8‡
p (strain)	‡	†	*
Incipient rearing			
C57BL/6	140.8 ± 7.7	96.0 ± 8.9‡	96.0 ± 7.9‡
DBA/2	68.9 ± 3.8	103.7 ± 7.5‡	89.2 ± 5.2†
p (strain)	‡		
Wall-leaning			
C57BL/6	62.7 ± 3.7	64.0 ± 3.1	64.6 ± 4.0
DBA/2	51.5 ± 3.6	70.9 ± 6.8*	60.6 ± 4.3*
p (strain)	*		
Locomotion			
C57BL/6	467.7 ± 21.4	475.5 ± 27.1	481.5 ± 26.7
DBA/2	394.0 ± 27.7	406.9 ± 38.3	435.4 ± 37.5
p (strain)	*		
Floor-sniffing			
C57BL/6	160.2 ± 9.7	163.0 ± 10.2	144.0 ± 7.7
DBA/2	203.7 ± 6.3	226.2 ± 12.6	218.8 ± 11.4
p (strain)	‡	‡	‡
Object-sniffing			
C57BL/6	24.1 ± 1.8	24.1 ± 1.6	21.1 ± 1.4
DBA/2	16.8 ± 1.2	19.2 ± 1.8	15.2 ± 1.1
p (strain)	‡	*	†
Object-leaning			
C57BL/6	5.0 ± 1.2	6.3 ± 2.5	7.9 ± 1.8
DBA/2	3.7 ± 0.8	4.4 ± 0.7	2.4 ± 0.6
p (strain)			

Values are the mean ± S.E.M.; n=20 for each group. Probabilities as determined by Mann-Whitney U-tests; *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

sniffing, horizontal locomotion (line crossings), and sniffing at the floor.

Antiserum

Because of the localization of dynorphin B in the mossy fibers, the injections with its antiserum should be placed into the CA3 region. The antiserum was raised against synthetic dynorphin B (Bachem, Bubendorf, Switzerland) which had been coupled to thyroglobulin with carbodiimide. Radioimmunoassays showed this to be a C-terminal-directed antiserum with no cross-reactivity to leucine- and methionine-enkephalin.

Injection Procedures

Fifteen minutes prior to testing, the anti-dynorphin B antiserum was injected stereotaxically, under light ether anesthesia, into the left dorsal hippocampus (total injection volume 0.5 μl). The 26S-gauge needle was introduced by piercing the skin, the skull, and the cerebral cortex. Using split litters, two dilutions of the antiserum were chosen for the experimental groups (n=20 for each): 1/226 and 1/113, having binding capacities of 9 and 18 pg of dynorphin B, respectively. Since our RIA determinations revealed that one hippocampus contains about 180 pg of dynorphin B (unpublished), it is estimated that these antiserum dilutions can inactivate 5 to 10% of the opioid. As control groups (n=20), animals injected with preimmune serum (1/113) were used. It seems most unlikely that under the experimental conditions the rabbit antiserum would differ from the preimmune serum with respect to hormones that could affect the behavior of the experimental mice. To check the correct placement of the injections, Evans blue (0.3%) was added to the fluids. After the behavioral observations the brains were fixed by transcardial perfusion with 4% paraformaldehyde and cryostat sections were examined. Only animals in which the dye was limited to the CA3 region of the hippocampus (61% of the cases) were included in the experiment. We have been very strict in applying this criterion. Behavioral influences of the injection procedure *per se* have been reported upon earlier; these do not to any marked extent detract from the strain differences [10].

RESULTS

Table 1 shows that both doses of the antiserum enhanced

TABLE 2

EXPLORATORY ACTS OBSERVED IN DBA/2 AND C57BL/6 MICE INJECTED WITH CONTROL SERUM AND WITH TWO DILUTIONS OF ANTI-DYNORPHIN B ANTISERUM: DATA OBTAINED AFTER (PARTLY) MISDIRECTED INJECTIONS

	Control	1/226	1/113
Rearing			
C57BL/6	36.3 ± 6.0	43.1 ± 8.6	40.4 ± 5.9
DBA/2	32.5 ± 4.0	45.9 ± 7.3	40.5 ± 6.6
p (strain)			
Incipient rearing			
C57BL/6	112.1 ± 4.6	101.9 ± 12.7	104.2 ± 7.2
DBA/2	76.9 ± 5.2	93.2 ± 5.9	83.3 ± 10.8
p (strain)	‡		
Wall-leaning			
C57BL/6	65.9 ± 7.6	66.4 ± 7.4	78.5 ± 6.5
DBA/2	62.0 ± 6.7	59.9 ± 7.2	57.9 ± 8.4
p (strain)			*
Locomotion			
C57BL/6	453.2 ± 30.4	457.1 ± 45.4	515.9 ± 33.2
DBA/2	384.1 ± 18.6	394.0 ± 40.2	411.9 ± 58.9
p (strain)			*
Floor-sniffing			
C57BL/6	153.7 ± 9.2	163.7 ± 9.3	161.9 ± 9.0
DBA/2	187.2 ± 8.8	238.2 ± 9.5‡	215.6 ± 13.0
p (strain)	†	‡	†
Object-sniffing			
C57BL/6	22.7 ± 2.2	20.7 ± 3.7	23.6 ± 1.4
DBA/2	19.0 ± 2.3	17.1 ± 1.8	18.9 ± 2.4
p (strain)			
Object-leaning			
C57BL/6	9.1 ± 2.7	13.0 ± 4.4	13.5 ± 2.5
DBA/2	6.2 ± 1.3	4.3 ± 1.4	5.1 ± 1.8
p (strain)			†

Values are the mean ± S.E.M.; n=9–17. Probabilities as determined by Mann-Whitney U-tests; *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

rearing significantly in strain DBA/2, whereas strain C57BL/6 responded with significant reductions. The strain difference originally present between the normal serum controls was reversed after these treatments. As for incipient rearing, such opposite treatment effects eliminated the original strain difference. The antiserum did not reduce wall-leaning in C57BL/6 but it did increase this behavior in DBA/2, leading to the disappearance of the strain difference. With regard to locomotor activity and floor-sniffing, the treatment effects also tended to go in opposite directions, but not significantly, and for object-sniffing and object-leaning there was no such tendency.

DISCUSSION

Evidently, genetic influences are important in hippocampally mediated behavioral responses to novelty and we have capitalized on this. Partial inactivation of hippocampal dynorphin B, released in mice exposed to a novel environment, caused the reversal or elimination of the differences between the two inbred strains, particularly as far as the vertically-oriented exploratory acts are concerned. The significance of this finding is strengthened by the fact that these opposite effects were not observed at all in the groups (n=9–17) discarded because of partly or entirely misdirected microinjections (see Table 2), i.e., the treatment effects are specific to CA3. Hence, the hypothesis of a genotype-dependent disinhibitory function of hippocampal dynorphin B in the regulation of mouse exploration, as outlined above, is strongly supported. In strain C57BL/6, the presumptively balanced dynorphinergic action seems to be upset by antiserum administration. For strain DBA/2, in contrast, an over-release of the opioid peptide apparently can be neutralized in part by treatment with diluted antiserum, leading to higher exploration rates.

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