EFFECT OF DES-1-TYROSINE-γ-ENDORPHIN ON [^{3}H]DOPAMINE AND [^{3}H]-γ-AMINOBUTYRIC ACID RELEASE FROM STRIATAL SYNAPTOSOMES

A. Yu. Shemanov, G. I. Kovalev,
A. V. Prikhozhan, and K. S. Raevskii

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Reports of the pharmacological properties and possible physiological role in the CNS of some fragments of β-endorphin of endogenous origin have recently been published [12]. One such fragment is des-1-tyrosine-γ-endorphin (DTGE), which exhibits several properties which resemble those of neuroleptics [6]. It has been suggested that an essential role in the mechanism of the pharmacological action of DTGE may be played by its influence on the dopaminergic systems of the brain and, in particular, on the rate of dopamine biosynthesis [2, 5]. Considering the complexity of the structural and functional organization of the mechanisms responsible for control of dopamine biosynthesis in the striatum and the important role of presynaptic mediator transport in these processes [8, 10], it was decided to study whether the presynaptic release of [^{3}H]dopamine ([^{3}H]-DA) and [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) is modified by the presence of DTGE. By carrying out the experiments on superfused synaptosomes *in vitro* it was possible to rule out involvement of postsynaptic regulation mechanisms.

EXPERIMENTAL METHOD

To study release of tritium-labeled mediators the technique of superfusion of the "coarse" synaptosome fraction [9] isolated from the brain of male Wistar rats weighing 180-200 g was used. The animals were decapitated, the striatum was removed quickly in the cold as described in [7] and homogenized in ten volumes of 0.32 M sucrose (pH 7.4, 0.2 mM EDTA), and the homogenate was centrifuged at 1000g for 10 min. The supernatant was recentrifuged at 20,000g for 25 min. The residue of "coarse" synaptosomes (fraction P2) was suspended in 0.32 M sucrose (pH 7.4, 0.2 mM EDTA). The protein concentration in the resulting suspension of synaptosomes was 7-10 mg/ml. After 30 min 50 μl of suspension was added to incubation medium (pH 7.4), in a volume of 1 ml, of the following composition: 124 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM D-glucose, 20 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 0.0125 mM nialamide (in experiments with [^{3}H]-DA) or 0.2 mM aminoacetic acid (in experiments with [^{3}H]-GABA). The label was added to the sample either immediately before addition of the synaptosomes (experiments with [^{3}H]-DA) or 2 min after their addition (experiments with [^{3}H]-GABA). The final concentration of [^{3}H]-DA was 0.1 μM and of [^{3}H]-GABA 0.1 mM. The samples were incubated at 37°C for 10 min ([^{3}H]-DA) or 5 min ([^{3}H]-GABA), after which the protein was precipitated on a Whatman GF/C filter, fixed in a thermostatically controlled chamber. The synaptosomes were washed three times with 5 ml of incubation medium each time, at the top speed of a peristaltic pump, after which they were superfused for 10 min with normal buffer at a speed of 0.6 ml/min. During the next 5 min the test substance was added to normal buffer (to study the effect of the peptides on spontaneous release) or to buffer containing 30 mM KCl (to study stimulated release), after which normal buffer was used again (5 min). Buffer containing 30 mM KCl was prepared by equimolar replacement of NaCl. One-minute fractions were collected directly into scintillation flasks. To all flasks 8 ml of Bray's scintillation solution [3] was added. Radioactivity was measured with an Intertechnique SL4000 counter, allowing for residual radioactivity on the filter. Stimulation of mediator release was expressed as a percentage above the basal level without stimulation, taken as 100. The results were subjected to statistical analysis and compared by Student's t test. The following reagents were used: [7, 8-^{3}H]dopamine (47 Ci/mmole, from Amersham Corporation, England) and [2,3-^{3}H]-GABA (16.2 Ci/mmole, from the All-Union "Izotop"

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TABLE 1. Effect of DTGE and TAE on [^{3}H]-DA Release From Striatal Synaptosomes of the Rat Brain Induced by a Depolarizing Concentration of Potassium Ions (30 mM) (M ± S.E.M.)

Experimental conditions	Stimulation of [^{3}H]-DA release, percent		Percent of control
Control	80,2±4,4	(13)	100,0
DTGE (10^{-6}M)	63,1±5,8*	(7)	78,7*
DTGE (10^{-4}M)	74,0±5,6	(5)	92,3
TAE (10^{-6}M)	77,3±2,8	(3)	96,4
TAE (10^{-4}M)	81,4±6,6	(4)	101,5

Legend. Number of experiments given in parentheses. *P < 0.05 compared with control (Student's t test)

TABLE 2. Effect of DTGE and TAE on [^{3}H]-GABA Release From Striatal Synaptosomes of Rat Brain Induced by a Depolarizing Concentration of Potassium Ions (30 mM) (M ± S.E.M.)

Experimental conditions	Stimulation of [^{3}H]-GABA release, percent		Percent of control
Control	53,0±4,5	(7)	100,0
DTGE (10^{-6}M)	52,5±7,6	(6)	99,1
DTGE (10^{-4}M)	60,0±8,7	(4)	113,2
TAE (10^{-6} M)	57,7±4,7	(2)	108,9
TAE (10^{-4}M)	48,5±8,8	(4)	91,5

Legend. Number of experiments given in parentheses.

Combine, USSR). The peptides were generously supplied by M. I. Titov (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR).

EXPERIMENTAL RESULTS

DTGE in a concentration of 10^{-6} M inhibited release of [^{3}H]-DA from striatal synaptosomes induced by a depolarizing concentration (30 mM) of potassium ions (Table 1). It must be emphasized that the character of the effect observed agreed with the hypothesis that the peptide has a modulating influence on dopaminergic transmission and with data published previously on the effect of DTGE on mediator liberation from striatal slices [11]. Since the possibility that the peptide may act on the postsynaptic stage of regulation of mediator release was ruled out in the experiments on synaptosomes, it can be concluded from these data that the inhibitory effect of DTGE on dopamine liberation from striatal slices is explained at least in part by its presynaptic action. . DTGE in a higher concentration had no effect on [^{3}H]-DA release (Table 1). DTGE likewise caused no change in [^{3}H]-GABA release from striatal synaptosomes induced by stimulation (Table 2). In similar experiments the effect of the opioid peptide Tyr-D-Ala-Gly-Phe-NH_2, a synthetic tetrapeptide analog of the enkephalins (TAE) [1], on release of [^{3}H]-DA and [^{3}H]-GABA from striatal synaptosomes was studied. In the concentrations tested the opioid peptide did not change the release of these mediators evoked by depolarization (30 mM KCl; see Tables 1 and 2). Neither peptide, studied in concentrations from 10^{-6} to 10^{-4} M, had any appreciable effect on spontaneous mediator release.

The results are evidence that the modulating effect of DTGE on dopamine release is evidently not mediated through its action on opiate receptors or through a change in GABA release from terminals on GABA-ergic striatal interneurons which, according to some evidence, may

modify dopamine release [10]. We likewise were unable to demonstrate any inhibitory effect of enkephalins on GABA release, discovered previously on synaptosomes of whole rat brain [4], possibly due to the absence of GABA-ergic nerve endings sensitive to these peptides in the striatum. It is interesting to compare the absence of effect of DTGE on dopamine release in the presence of a higher concentration of the peptide (10^{-4} M) with the decrease in cataleptogenic activity of DTGE when its dose is increased above the optimal level [2]. It can be tentatively suggested that DTGE, like neuroleptics, has a cataleptogenic action and modulates dopaminergic transmission in the striatum. Dependence of the effect of DTGE on dopamine liberation on its concentration revealed by these experiments could serve as one explanation for the abnormal dependence of the behavioral effect of this peptide on its dose.

LITERATURE CITED

1. V. V. Zakusov, V. M. Bulaev, O. N. Chichenkov, et al., Byull. Éksp. Biol. Med., No. 2, 171 (1981).
2. K. S. Raevskii, A. Yu. Shemanov, and V. S. Kudrin, Farmakol. Toksikol., No. 5, 5 (1982).
3. A. G. Bray, Anal. Biochem., 1, 279 (1960).
4. M. J. Brennan, R. C. Cantrill, and B. A. Wylie, Life Sci., 27, 1097 (1980).
5. D. De Wied, Trends Neurosci., 2, 79 (1979).
6. D. De Wied, G. L. Kovacs, B. Bohus, et al., Eur. J. Pharmacol., 49, 427 (1978).
7. J. Glowinski and L. L. Iversen, J. Neurochem., 13, 655 (1966).
8. B. A. McMillen and P. A. Shore, Naunyn-Schmiedeberg's Arch. Pharmakol., 313, 39 (1980).
9. M. Raiteri, F. Angelini, and G. Levi, Eur. J. Pharmacol., 25, 414 (1974).
10. W. Reimann, Naunyn-Schmiedeberg's Arch. Pharmakol., 316, Suppl. No. 265, R67 (1981).
11. H. Schoemaker and V. J. Nickolson, Life Sci., 27, 1371 (1980).
12. J. Verhoef, V. M. Wiegant, and D. De Wied, Brain Res., 231, 454 (1982).

DISSIMILAR EFFECTS OF METHIOTHEPIN AND PIRENPERONE ON BEHAVIORAL EFFECTS OF APOMORPHINE

É. É. Vasar and L. Kh. Allikmets

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There is evidence in the literature that a change in activity of serotoninergic processes leads to dissimilar changes in the behavioral effects of apomorphine. Small doses of serotoninomimetics appreciably potentiate apomorphine stereotypy, but destruction of serotoninergic neurons or blockade of serotonin receptors reduces the intensity of stereotyped behavior [4, 6]. Serotonin antagonists and agonists have opposite effects of apomorphine aggressiveness compared with stereotyped behavior. Serotonin antagonists potentiate, whereas agonists inhibit aggressive behavior [7].

It is shown in this paper that on prolonged administration of apomorphine the sensitivity of serotonin receptors linked with aggressive behavior is increased whereas the sensitivity of other receptors, linked with stereotyped behavior, is depressed.

EXPERIMENTAL METHOD

Behavioral experiments were carried out on 150 male Wistar rats weighing 270-320 g. The animals were divided into groups, with 10 to 12 rats in each group. Apomorphine was injected subcutaneously in a dose of 0.5 mg/kg twice a day for ten days. Serotonin antagonists pirenperone (from Jansen Pharmaceutica, Belgium) in doses of 0.07 to 0.3 mg/kg and methiothepin

Departments of Physiology and Pharmacology, Tartu University.

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