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PRE-DECAPITATION STATE OF AROUSAL OF RATS PREDETERMINES THE EFFECT OF DES-TYR^{1}-γ-ENDORPHIN ON DOPAMINE RELEASE FROM NUCLEUS ACCUMBENS SLICES IN VITRO

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Summary

The non-opiate β-endorphin fragment des-Tyr^{1}-γ-endorphin (DTγE) had a decreasing effect on K^{+}-induced release of tritiated dopamine from nucleus accumbens slices in vitro, when tissue was used of rats which prior to decapitation were in a state of low arousal. When nucleus accumbens tissue was used of rats which were mildly stressed by exposure to a novel environment prior to decapitation, this effect was absent, while an enhancing effect of DTγE became evident on basal dopamine efflux. This latter effect resembled that of haloperidol, which dose-dependently enhanced basal dopamine efflux in vitro. Exposure of rats to ether vapor shortly before decapitation abolished both these in vitro effects of DTγE. The results are interpreted as indicating that the quality of the modulating effects of DTγE on dopamine release from dopaminergic neurons projecting to the nucleus accumbens is depending on the state of activity of these neurons, which, in its turn, is a reflection of the state of arousal of the rats

The non-opiate β-endorphin fragments des-Tyr^{1}-γ-endorphin (β-endorphin-(2-17); DTγE) and des-enkephalin-γ-endorphin (β-endorphin-(6-17); DEγE) have been shown to have neuroleptic-like effects (1-3). Various approaches have been used to obtain information as to whether γ-type endorphins exert their neuroleptic-like effect via an interaction with brain dopamine systems. On basis of the results of a series of behavioral experiments, in which DTγE and DEγE were microinjected into the nucleus accumbens of the rat, Van Ree and De Wied (3) have postulated that γ-type endorphins affect dopaminergic activity in this brain region by modulating self-inhibitory dopamine receptor systems. Although various reports have dealt with the presence or absence of effects of γ-type endorphins on brain dopamine metabolism (4-9), neurochemical data on effects on nucleus accumbens dopamine are particularly few in number. According to Schoemaker and Nickolson (7) K^{+}-induced release of dopamine from cortical slices and from slices of caudate nucleus and nucleus accumbens in vitro is slightly but significantly decreased in the presence of 6×10^{-8} M DTγE, an effect which differs from that of the classical neuroleptic haloperidol. We, however, found no effect of intracerebroventricularly (i.c.v.) administered DTγE on α-MPT-induced disapearance of dopamine in the nucleus accumbens (4,5).

While attempting to replicate the results of Schoemaker and Nickolson (7) we found a small, but significant effect of DTγE on K^{+}-induced dopamine release in vitro from nucleus accumbens slices in some, but not all experiments. We decided, therefore, to investigate in more detail the influence of the pre-decapitation handling of the rats on this in vitro effect of DTγE.

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Methods

Male Wistar rats, weighing 150-200 g, were subjected to one of five different pre-decapitation procedures:
A) No handling, novel environment: on the morning of the experiment the rats were transferred from the animal facilities to a room in another part of the building and decapitated.
B) Handling, novel environment: as under A), but prior to transfer the rats were extensively handled twice a day for 5 days.
C) Handling, no novel environment: rats were handled as under B), but they were decapitated without being transferred to another room.
D) Pentobarbital anesthesia: handling as under B); on the morning of the experiment the rats were anesthetized with pentobarbital (Nembutal, 50 mg/kg, i.p.) and decapitated 30 min later.
E) Ether stress: handling as under B); on the morning of the experiment the rats were exposed to ether vapor for 2 min and decapitated 5 or 15 min later.

Dopamine release from nucleus accumbens slices in vitro was measured according to Stoof et al. (10). Briefly, after decapitation of the rats the brains were rapidly taken out of the skull. Subsequently the nucleus accumbens was dissected from a 2 mm section. The dissected tissue was then chopped in pieces with dimensions of approximately 0.3 x 0.3 x 2 mm with a McIlwain tissue chopper. The slices were preincubated in Krebs-Ringer bicarbonate medium, pH 7.2-7.4 (in mM: NaCl 119; KCl 4.75; KH_2PO_4 1.17; $MgSO_4.7\ H_2O$ 1.19; $NaHCO_3$ 25.5; $CaCl_2$ 1.27; glucose 11) for 5 min and incubated in this medium containing [2,5,6-^{3}H]dopamine (specific activity 10 Ci/mmol; Amersham) at a final concentration of $2x10^{-7}$ M for 15 min. After rinsing, 5-6 slices were transferred to each of 16 chambers (volume 0.5 ml) of a superfusion apparatus and superfused at 37°C with medium at a rate of 0.25 ml/min. After 40 min of superfusion eight 10-min fractions were collected. Depolarization-induced release of ^{3}H-dopamine was effected by changing to medium containing 20 mM K^+ for 5 min. This was done at t = 60 and t = 100 min. Peptide or haloperidol was present in the superfusion medium from t = 70 min up till the end of the run.

The radioactivity remaining in the slices was extracted with 0.1 N HCl. Radioactivity in 1 ml aliquots of the collected fractions and in the tissue extracts was determined by liquid scintillation spectrometry. The release of radioactivity was calculated as fractional rate. S_1 and S_2 represent the release of radioactivity in excess of basal efflux resulting from stimulation with 20 mM K^+. The ratio S_2/S_1, i.e of the percentage of radioactivity released during the second and first stimulation, was calculated for both control slices and slices exposed to peptide or to haloperidol. Fractions Sp_2 and Sp_8 represent the basal efflux in the second and 8th fraction respectively. The ratio of the percentage released in Sp_8 and Sp_2 was calculated for both control slices and slices exposed to peptide or to haloperidol.

Results

As can be seen from Table 1, DTγE either caused a slight, but significant decrease in K^+-induced dopamine release or enhanced the spontaneous efflux of dopamine from nucleus accumbens slices in vitro or had no effect on either of these paradigms, depending on the pre-decapitation state of arousal of the rats. The effect of DTγE on K^+-induced dopamine release is similar to that previously reported by Schoemaker and Nickolson (7). In our experiments this effect was only observed, when nucleus accumbens tissue was used from rats which had been subjected to an extensive handling procedure and when the tissue came from rats which had been anesthetized with pentobarbital (Table 1, C & D). In those experiments in which nucleus accumbens tissue was used of rats which had transferred to a novel environment prior to decapitation, no effects were observed of DTγE on K^+-induced dopamine release (Table 1, A & B). Consistent in-

TABLE 1

Effects of des-Tyr^1-γ-endorphin (DTγE) on K^+-induced and basal release of ^{3}H-dopamine from nucleus accumbens slices *in vitro* after various pre-decapitation handling procedures

Pre-decapitation handling procedure	K^+-induced ^{3}H-DA release (S_2/S_1)		Basal ^{3}H-DA release (Sp_8/Sp_2)	
	Controls	DTγE[a]	Controls	DTγE[a]
A) No handling, novel environm.				
Experiment 1	0.51±0.05[b]	0.54±0.07	0.41±0.06	0.67±0.04*
B) Handling, novel environm.				
Experiment 1	0.51±0.05	0.50±0.07	0.42±0.03	0.68±0.06*
Experiment 2	0.56±0.04	0.62±0.04	0.59±0.02	0.79±0.04*
C) Handling, no novel environm.				
Experiment 1	0.81±0.03	0.71±0.01*	0.93±0.02	0.87±0.03
Experiment 2	0.67±0.03	0.57±0.01*	0.80±0.03	0.77±0.02
Experiment 3	0.76±0.04	0.64±0.02*	0.82±0.05	0.75±0.02
D) Pentobarbital anesthesia				
Experiment 1	0.59±0.02	0.47±0.03*	0.94±0.02	0.80±0.03*
Experiment 2	0.89±0.05	0.75±0.03*	0.77±0.05	0.78±0.03
E) Ether stress				
2 min ether, † at t= 5'	0.70±0.02	0.63±0.04	0.81±0.01	0.85±0.01
2 min ether, † at t=15'	0.65±0.04	0.73±0.14	0.52±0.06	0.52±0.03

Nucleus accumbens slices, preloaded with ^{3}H-dopamine (^{3}H-DA) were superfused with Krebs-Ringer bicarbonate medium, pH 7.2-7.4. Eight 10-min fractions were collected at a superfusion rate of 0.25 ml/min. Depolarization-induced ^{3}H-DA release was produced by changing to medium containing 20 mM K^+ for 5 min, starting at the beginning of the 3rd and 6th fraction. The release of radioactivity per fraction was calculated as fractional rate of total radioactivity. For details concerning the calculation of the ratios S_2/S_1 and Sp_8/Sp_2 see text.

a) DTγE was present in the superfusion medium in a concentration of 6×10^{-8} M starting from the 4th fraction.

b) mean±S.E.M. (n = 8, except for Pentobarbital anesthesia, Exp. 1, where n = 4)

* $P<0.05$ for differences with controls (Student's t-test, two-tailed).

creases in basal dopamine efflux, however, became evident under these circumstances (Table 1, A & B). This effect on basal dopamine efflux was observed irrespective whether the rats prior to transfer had been handled or not. Severe stress, consisting of exposure of the rats to ether vapor prior to decapitation, abolished also this effect of DTγE on dopamine release from nucleus accumbens slices *in vitro* (Table 1, E).

As can be seen from Table 2, A & B, both the effect of DTγE on basal dopamine efflux from nucleus accumbens slices from non-handled rats and the effect of DTγE on K^+-induced dopamine release from slices of pentobarbital anesthetized rats exhibit a non-linear, bell-shaped dose-response curve, with a maximal effect at a concentration of 6×10^{-8} M, which also was the concentration used in all experiments of which the results are shown in Table 1.

Table 2, C, shows that the increasing effect of haloperidol on basal dopamine release is dose-dependent in the concentration range of 10^{-7} - 5×10^{-6} M. Effects of haloperidol on K^+-induced dopamine release were not calculated, since

TABLE 2

Effect of des-Tyr^1-γ-endorphin (DTγE) and haloperidol on K^+-induced and basal release of ^{3}H-dopamine (^{3}H-DA) from nucleus accumbens slices in vitro

	K^+-induced ^{3}H-DA release (S_2/S_1)	Basal ^{3}H-DA release (Sp_8/Sp_2)	
A) DTγE, no handling [a]			
0 M	0.66±0.06	0.96±0.04 [b]	(12)
6×10^{-9} M	0.66±0.02	1.33±0.08*	(12)
6×10^{-8} M	0.77±0.07	1.39±0.11*	(12)
6×10^{-7} M	0.68±0.05	1.16±0.05*	(8)
B) DTγE, pentobarbital anesthesia [a]			
0 M	0.80±0.03	0.84±0.03	(8)
6×10^{-9} M	0.75±0.02	0.75±0.03	(8)
6×10^{-8} M	0.58±0.06*	0.88±0.06	(8)
6×10^{-7} M	0.84±0.02	0.74±0.04	(8)
C) Haloperidol [a]			
0 M	not calculated	0.94±0.04	(8)
10^{-7} M	" "	1.58±0.03*	(6)
5×10^{-7} M	" "	1.71±0.08*	(6)
10^{-6} M	" "	2.29±0.17*	(5)
5×10^{-6} M	" "	4.76±0.44*	(6)

a) DTγE or haloperidol were present in the superfusion medium starting from the 4th fraction (for further details see text).

* $P<0.05$ for difference with controls (Student's t-test, two-tailed).

in the dose range employed the effect of haloperidol on basal efflux interferes with that on stimulated release.

Discussion

It has been suggested that the nucleus accumbens has a filtering or gating function (11,12). Mesolimbic dopamine projections to the nucleus accumbens supposedly play an important role in this postulated gating mechanism and are assumed to serve as an interface between limbic and motor systems (13). The present results indicate that the state of arousal of the rat at the time of decapitation predetermines the quality of the effect which subsequently DTγE can exert in vitro on the release of dopamine from nucleus accumbens slices. The present experimental design did not enable the visualization of differences in activity of release mechanisms as such as a consequence of the state of arousal of the rats. Thierry et al. (14) in in vivo experiments have found that stress selectively increases the utilization of dopamine in the nucleus accumbens. The nucleus accumbens abounds with classical transmitters as well as with neuropeptides (15). Among the latter are substance P, TRH, somatostatin, enkephalins, VIP, CCK, α-MSH and neurotensin (15). Both β-endorphin- and γ-endorphin-like

immunoreactivity have been observed in the nucleus accumbens, albeit in relatively low concentration (16). Recently, Starr (17) reported that a variety of neuropeptides, including substance P, somatostatin, CCK and neurotensin, is able to alter either basal efflux or the K^+-induced release, or both, of dopamine from striatal slices *in vitro*. Starr, based on these findings, suggested that modulation of dopaminergic neuronal activity may be a common feature of many neuropeptides following their endogenous release (17). We previously have observed that mediobasal hypothalamic catecholamine synthesizing mechanisms continue to operate *in vitro* at pre-decapitation levels of activity (18,19). The present finding that DTγE's effect on dopamine release *in vitro* from nucleus accumbens slices differs depending on the pre-decapitation state of arousal of the rats can be interpreted as indicating that this same holds for the state of modulation of the mechanisms responsible for the release of dopamine from dopaminergic neurons projecting to the nucleus accumbens.

We can only speculate as to how exactly DTγE influences dopamine release in the nucleus accumbens. Using α-MPT-induced disappearance of dopamine as a paradigm, in previous experiments we have found that DTγE enhanced dopamine utilization in a number of brain regions innervated by the so-called intra-diencephalic dopamine systems, but failed to do so in the caudate nucleus and nucleus accumbens (4,5). In this respect DTγE acted differently from haloperidol, which, in the same experimental design, enhanced dopamine utilization in all brain regions, including the caudate nucleus and nucleus accumbens (20). Treatment with α-MPT, however, is known to be a substantial stress (21,22) and, therefore, might abolish the more subtle effects of DTγE on nucleus accumbens dopamine. As to the decreasing effect of DTγE on K^+-induced dopamine release from nucleus accumbens slices from non-stressed rats found in the present study, this too differs from that of haloperidol, as has previously also been reported by Schoemaker and Nickolson (7).

For striatal slices it has been shown that haloperidol in low concentrations (nmolar range) increases K^+-induced dopamine release *in vitro*, whereas in high concentrations (μmolar range) it enhances the spontaneous efflux of dopamine (23-25). The latter effect of haloperidol on basal dopamine efflux is also present in nucleus accumbens slices *in vitro* (see Table 2). It is interesting to note that the effect of haloperidol on basal dopamine efflux is similar to that of DTγE observed after mild arousal of the rats prior to decapitation. Table 2 shows that haloperidol dose-dependently increases basal dopamine efflux, exhibiting a dramatic effect at high concentrations. The dose-response curve for DTγE, however, is non-linear, bell-shaped, and suggests that the neuropeptide operates in a rather narrow concentration range. In contrast to neuroleptics, γ-type endorphins do not displace ^{3}H-spiperone from its binding sites *in vitro* (26-28). Though Pedigo et al. (29) have reported that DTγE did displace ^{3}H-spiperone *in vivo* from hypothalamus, striatum and nucleus accumbens, more recent studies have yielded negative results (28,30). It thus seems unlikely that γ-type endorphins interact directly with the dopamine/neuroleptic receptors. The present results are in support for the hypothesis that γ-type endophins have neuroleptic-like characteristics. They also indicate that DTγE acts on nucleus accumbens dopamine neurons, though with a mechanism of action which differs from that of neuroleptics.

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