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The neuroleptic-like peptide desenkephalin-γ-endorphin does not antagonize the dopamine receptor agonist-induced inhibition of the release of [^{3}H]dopamine from rat nucleus accumbens slices in vitro

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In rats, the non-opioid β-endorphin (βE) fragment desenkephalin-γ-endorphin (DEγE, βE_{6-17}) antagonizes the hypomotility induced by a small dose of dopamine (DA) receptor agonists. It has been suggested that DEγE might act in this respect by a direct or indirect blockade of presynaptically located DA receptors in the nucleus accumbens, thereby causing an increase of DA release. Therefore in the present study the effect of DEγE was examined on DA receptor agonist-induced inhibition of the electrically evoked release of previously accumulated [^{3}H]DA from rat nucleus accumbens slices in vitro. The DA receptor agonists apomorphine, LY 171555 and *n,n*-di-*n*-propyl-7-hydroxy-2-aminotetralin (DP-7-AT) inhibited in a concentration-dependent manner the electrically evoked release of [^{3}H]DA. The selective D_2 receptor antagonist (–)-sulpiride blocked the effects of apomorphine, corroborating that the DA receptor involved is of a D_2 type. DEγE was tested at several concentrations (10^{-9}–10^{-6} M) and under various experimental conditions. DEγE, by itself, did not affect either the electrically stimulated or the basal release of [^{3}H]DA. The inhibiting effect of DA receptor agonists was slightly reduced by DEγE, but this effect was present in some experiments only. It is concluded that DEγE does not function as an antagonist for the DA receptor mediating DA release and that the interaction observed in behavioural experiments between DA agonists and DEγE does not occur at the level of this receptor.

INTRODUCTION

In rats, the non-opioid β-endorphin (βE) fragments des-Tyr[1]-γ-endorphin (DTγE, βE_{2-17}) and desenkephalin-γ-endorphin (DEγE, βE_{6-17}) induce effects which are similar to those of neuroleptics in a number of behavioural test procedures[2,3,21]. Since the major characteristic of neuroleptics is their dopamine (DA) receptor-blocking activity, the interaction between DEγE, the shortest γ-endorphin fragment with neuroleptic-like activity, and brain DA has been investigated in detail during the last few years. A series of studies dealing with systemic treatment has revealed that DEγE antagonizes the hypomotility induced by small doses of the DA receptor agonist apomorphine and does not affect the behavioural effects observed after injection with relatively large doses of this drug[23]. Apparently, the neuroanatomical substrate for this antagonism is the nucleus accumbens, since local injection of apomorphine into this brain region also induces hypomotility[22] and since this apomorphine-induced hypomotility could be antagonized by local administration of DEγE as well as by the DA receptor antagonists haloperidol and sulpiride[24]. Moreover, the hypomotility induced by systemic administration of apomorphine could be blocked by local injections of DEγE and neuroleptics into the nucleus accumbens[14]. It has been hypoth-

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esized that this action of DEγE is due to interference with DA receptor systems located presynaptically and sensitive to low doses of both dopamine agonists and antagonists[25]. In accordance with this postulate we have found that the hypomotility induced by injection of apomorphine into the nucleus accumbens was not present following destruction of the DA terminals in this brain area by the neurotoxin 6-hydroxydopamine.

Results of behavioural studies have suggested that DEγE might act by a direct or indirect blockade of presynaptically located DA receptors, thereby causing an increase of DA release[24]. This possibility was investigated in the present study, in which we examined the effects of DEγE on DA receptor agonist-induced inhibition of the release of [^{3}H]DA in vitro. It has recently been demonstrated that not only in the neostriatum but also in the nucleus accumbens DA receptor agonists are able to inhibit the depolarization-induced release of previously accumulated radioactive DA[13]. In the present series of experiments we examined the effects of DEγE itself and DEγE in combination with DA agonists, i.c. apomorphine, the D_2 agonist LY 171555 and the preferentially presynaptic agonist *n,n*-di-*n*-propyl-7-hydroxy-2-aminotetralin (DP-7-AT)[29], on the electrically evoked release of [^{3}H]DA from rat nucleus accumbens slices to establish the possible existence of interactions with DA autoreceptors. The obtained data suggest that the interaction between DEγE and DA receptor agonists as observed in behavioural experiments, is not reflected at the level of the radiolabelled DA release in vitro.

MATERIALS AND METHODS

Preparation of slices

Male Wistar rats, weighing 170–200 g, were killed by decapitation. Prior to decapitation the rats were handled twice daily for 5 days. The brains were excised rapidly and the nucleus accumbens dissected bilaterally according to the method of Horn et al.[5]. The dissected pieces of tissue were minced by passing them twice through a McIlwain tissue chopper (micrometer setting 300 μm). Prior to the second pass the surface was rotated 90°.

The resulting slices measured approximately 2 × 0.3 × 0.3 mm and were collected in Krebs–Ringer bicarbonate (KRB) medium containing (in mM) NaCl 121, KCl 1.87, KH_2PO_4 1.17, $MgSO_4$ 1.17, $CaCl_2$ 1.20, $NaHCO_3$ 25 and D(+)-glucose 10. The medium was kept under a constant atmosphere of 95% O_2 and 5% CO_2.

[^{3}H]DA release studies

The tissue slices were washed twice in a few millilitres of fresh medium and then preincubated in a shaking waterbath for 15 min in 10 ml KRB medium at 37 °C under a constant flow of 95% O_2 and 5% CO_2. Subsequently the slices were washed in a few millilitres of fresh medium (37 °C); 2 ml of fresh medium were added containing 2.5 μCi [^{3}H]DA (spec. act. 49 Ci/mmol). The slices were incubated for 15 min, resulting in a selective labelling of dopaminergic nerve terminals. At the end of this incubation period, the tissue slices were washed with medium (1 ml). Aliquots of approximately 5 mg (wet wt.) were then transferred to each of the 24 chambers (volume 0.2 ml) of a superfusion apparatus maintained at 37 °C, and superfused at a rate of 0.25 ml medium/min.

The release of labelled neurotransmitter was stimulated electrically by exposing the tissue to electrical block pulses of biphasic polarity (1 Hz, 24 mA, 4 mS). Following a presuperfusion period of 45 min, 3 different experimental protocols were used. In the first protocol seven 15-min fractions were collected. During superfusion the tissue was exposed twice to electrical stimulation for 15 min, starting at t = 60 min (S_1) and t = 105 min (S_2) respectively. Drugs were added to the superfusion medium 10 min or 15 min prior to S_2.

In the second protocol the same time schedule was followed. Drugs were added to the superfusion medium 15 min prior to S_1 (DEγE or (–)-sulpiride) and 10 min prior to S_2 (apomorphine). In the third protocol four 15-min fractions were collected. The tissue was exposed once to electrical stimulation during a 15-min period starting at t = 60 min. Drugs were added to the medium at the start of the presuperfusion (t = 0 min, DEγE) and 20 min prior to stimulation (t = 40 min, DP-7-AT). All experiments, except those with DP-7-AT were performed in the presence of 0.03 mM EDTA-Na_2 in the superfusion medium in order to prevent oxidation of apomorphine. All superfusion media containing peptide were kept in plastic vials instead of glass vials in order to avoid adsorption

of peptide to glass. At the end of the experiment the remaining radioactivity was extracted from the tissue with 0.1 N HCl. Radioactivity in superfusion fractions and tissue extracts was determined by liquid scintillation counting.

Materials

[7,8-^{3}H]Dopamine was purchased from the Radiochemical Centre (Amersham), Titriplex III (EDTA-Na_2) from Merck and apomorphine hydrochloride (apomorphine) from Sandoz. LY 171555 (4,4a,5,6,7,8,8a,9-octahydro-5-*n*-propyl-2H-pyrazolo-3,4-g-quinoline) was kindly donated by Eli Lilly, DP-7-AT by Prof. Dr. A.S. Horn (State University of Groningen, The Netherlands), (–)-sulpiride by Delagrange, and DEγE (desenkephalin-γ-endorphin, βE_{6-17}) by Organon (Oss, The Netherlands).

Data analysis and statistics

The efflux of radioactivity during each 15-min collection period was expressed as the fraction of the radioactivity present in the slices at the beginning of the respective collection period (fractional rate of efflux). In order to calculate the electrically evoked neurotransmitter release the basal efflux of radioactivity (the sum of the fractional rates of the first and the fourth collection period for S_1 and the sum of the fractional rates of the fourth and the seventh collection period for S_2) was subtracted from the total overflow of radioactivity during the 15-min stimulation and the 15 min following stimulation. Thus, the electrically evoked release (S_1 and S_2) was then expressed as a percentage of the ^{3}H content of the slices at the start of the stimulation period. Finally, the ratio (S_2/S_1) of the percentages of radioactivity released by the second and first stimulation, respectively, was calculated. Effects of drugs were expressed as percentages of the mean control value for S_1 or S_2/S_1 respectively. In every experiment 4, 6 or 8 superfusion chambers were used as a control group. Statistical analysis of the data was performed using a one way analysis of variance (ANOVA) followed by the Newman–Keuls procedure in case the outcome revealed a significant effect ($P < 0.05$).

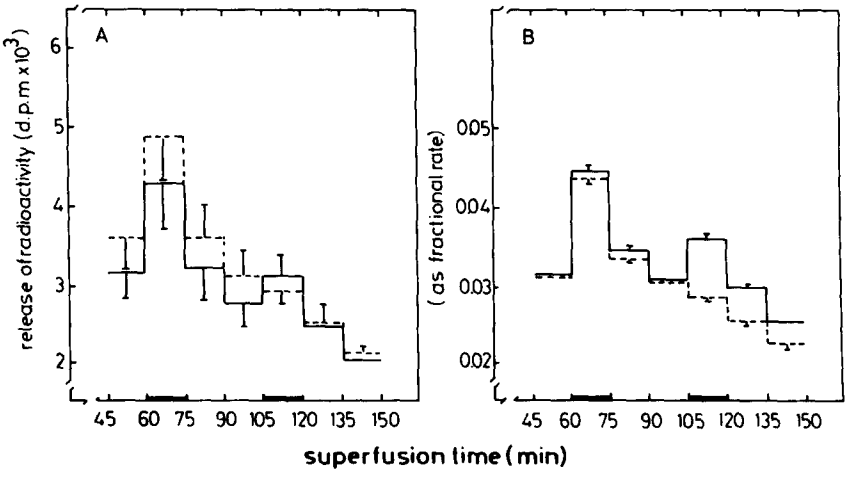

Fig. 1. Result of a typical experiment showing the effect of 10^{-8} M LY 171555 on the electrically evoked release of [^{3}H]DA from rat nucleus accumbens slices. A: the amount of radioactivity (measured dpm) released into the superfusion medium during experimental protocol 1 as described in Materials and Methods is plotted as a function of the superfusion time. The solid line across the top of each bar represents data (mean ± S.E.M.) from control superfusions; the broken line across the top of each bar represents data (mean ± S.E.M.) from tissue exposed to LY 171555 (10^{-8} M) starting at superfusion time t = 90 min. The amount of radioactivity released in the HCl fraction (which is not shown in the figure) was 80462 ± 9352 dpm in the absence and 95267 ± 10569 dpm in the presence of LY 171555. The period of time during which the tissue was exposed to electrical stimulation as described in Materials and Methods is indicated on the abscissa. B: mathematical transformation of the data presented in A. Using the data presented in A, the fractional rate of release of [^{3}H]DA was calculated using the protocol described in Materials and Methods. The release of radioactivity in excess of basal efflux resulting from electrical stimulation was calculated as the percentage of total radioactivity present at the onset of stimulation. For this calculation the total period in which the overflow was elevated above basal efflux was included. The ratio (S_2/S_1) of the percentages of radioactivity released as a result of the first and second stimulation respectively, was calculated for both control and drug-treated slices. Mean values for absolute release of [^{3}H]DA from control slices were 1.65 ± 0.11% during S_1 and 0.94 ± 0.04% during S_2 (n = 4); the control S_2/S_1 ratio was 0.58 ± 0.05 (mean ± S.E.M.).

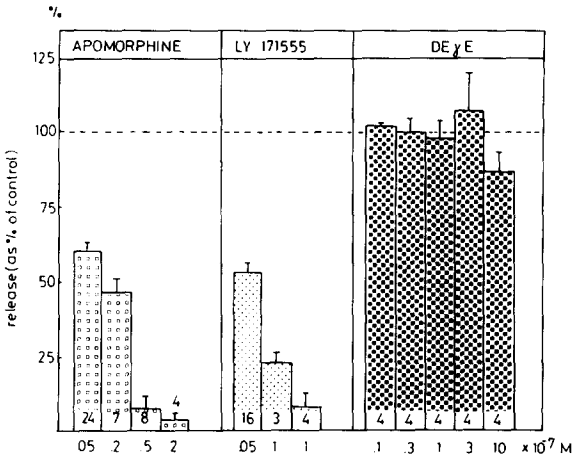

Fig. 2. Concentration dependency of the effect of DA receptor agonists and DEγE on the electrically evoked release of [^{3}H]DA from rat nucleus accumbens slices. Tissue was exposed twice to electrical stimulation. Apomorphine was added to the medium 10 min prior to the second stimulation and LY 171555 and DEγE 15 min prior to the second stimulation (protocol 1). The values represent mean percentage ± S.E.M. (n) of control. The number of observations is depicted in the columns.

RESULTS

Modulation of the electrically evoked release of [^{3}H]DA by apomorphine, LY 171555 and DEγE

Previously, the electrically evoked release of ^{3}H in excess of the spontaneous efflux of radioactivity has been shown to reflect the depolarization-induced exocytotic release of [^{3}H]DA[10,11,13]. The amount of radioactivity released from nucleus accumbens slices previously incubated with [^{3}H]DA is presented in Fig. 1A as a function of time. Fig. 1B shows the data of Fig. 1A following calculation of the fractional rate of release. The DA receptor agonists apomorphine and LY 171555 inhibited in a concentration-dependent manner the electrically evoked release of [^{3}H]DA from rat nucleus accumbens slices (Fig. 2). For both drugs half-maximal inhibition was achieved at approximately 5×10^{-9} M. The neuropeptide DEγE, at the concentrations 10^{-8}–10^{-6} M, did not affect either the electrically evoked release of [^{3}H]DA (Fig. 2) or the basal release (data not shown). DEγE, 10^{-6} M, did not influence the half-maximal inhibition of [^{3}H]DA of either DA receptor agonist (Fig. 3). There was a tendency towards reduction of the agonist-induced inhibition when DEγE was present in a lower concentration (10^{-7} M) (Fig. 3).

TABLE I

Interaction of DEγE and the DA receptor antagonist (–)-sulpiride with the apomorphine-induced inhibition of the electrically evoked release of [^{3}H]DA from rat nucleus accumbens slices

Tissue was exposed twice to electrical stimulation. DEγE or (–)-sulpiride were added to the superfusion medium at the start of the collection of the first fraction and apomorphine was added 50 min later, 10 min prior to the second stimulation (protocol 2). The values represent mean percentage ± S.E.M. of control. The number of observations was 4–8 per group. * Difference from control ($P < 0.05$, Newman–Keuls). ° Different from apomorphine ($P < 0.05$, Newman–Keuls).

Drug(s)	*Concentration (M)*	*[^{3}H]DA release (as % of control)*
Control		100.0 ± 2.3
Apomorphine	2×10^{-8}	46.4 ± 4.8*
Apomorphine + (–)-sulpiride	2×10^{-8} + 10^{-8}	70.8 ± 3.1*°
Apomorphine + (–)-sulpiride	2×10^{-8} + 10^{-7}	96.2 ± 0.6°
Apomorphine + DEγE	2×10^{-8} + 10^{-7}	41.4 ± 3.2*
(ANOVA: $F_{4,30} = 67.7$, $P < 0.001$)		
Control		100.0 ± 1.4
Apomorphine	5×10^{-9}	71.9 ± 1.4*
Apomorphine + DEγE	5×10^{-9} + 10^{-9}	74.6 ± 2.4*
Apomorphine + DEγE	5×10^{-9} + 10^{-8}	66.3 ± 3.3*
Apomorphine + DEγE	5×10^{-9} + 10^{-7}	87.9 ± 2.2*°
Apomorphine + DEγE	5×10^{-9} + 10^{-6}	74.8 ± 2.9*
(ANOVA: $F_{5,46} = 27.8$, $P < 0.001$)		
Control		100.0 ± 2.8
Apomorphine	10^{-8}	63.9 ± 3.0*
Apomorphine + DEγE	10^{-8} + 10^{-7}	69.4 ± 2.7*
(ANOVA: $F_{2,22} = 45.4$, $P < 0.001$)		

Interaction between apomorphine and (–)-sulpiride or DEγE

(–)-Sulpiride, when added to the superfusion medium 50 min prior to apomorphine, reversed in a concentration-dependent manner the half-maximal inhibition of the release of [^{3}H]DA induced by 2×10^{-8} M apomorphine (Table I).

In contrast to (–)-sulpiride, DEγE, tested at graded concentrations, was ineffective in reversing the effect of apomorphine, except that a slight, but statistically significant inhibiting effect of DEγE was found when 5×10^{-9} M apomorphine was combined with 10^{-7} M DEγE. When present in the superfusion medium alone, (–)-sulpiride and DEγE did not affect

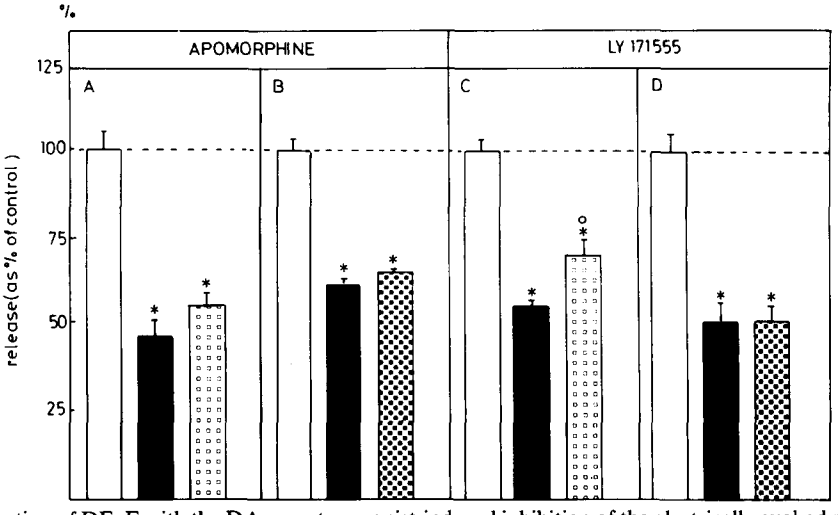

Fig. 3. Interaction of DEγE with the DA receptor agonist-induced inhibition of the electrically evoked release of [^{3}H]DA from rat nucleus accumbens slices. Tissue was exposed twice to electrical stimulation. DEγE (A and C: 10^{-7} M; B and D: 10^{-6} M) was added to the medium 15 min prior to the second stimulation. LY 171555 was added simultaneously with DEγE and apomorphine 5 min later than DEγE (protocol 1). The values represent mean percentage ± S.E.M. of control. Conditions: open column, control; black column, apomorphine or LY 171555 (5×10^{-9} M); squares, agonist + DEγE (10^{-7} M); checkerboard, agonist + DEγE (10^{-6} M). The number of observations was 3–8 per group. Results ANOVA: A: $F_{2,19} = 42.0$, $P < 0.001$; B: $F_{2,23} = 55.4$, $P < 0.001$; C: $F_{2,10} = 39.2$, $P < 0.001$; D: $F_{2,23} = 38.6$, $P < 0.001$. *Different from control ($P < 0.05$, Newman–Keuls). °Different from agonist without DEγE ($P < 0.05$, Newman–Keuls).

either the electrically stimulated release of [^{3}H]DA or the spontaneous efflux of radioactivity measured in the first collection period (data not shown).

Interaction between DP-7-AT and DEγE

The DA receptor agonist DP-7-AT inhibited the release of [^{3}H]DA from rat nucleus accumbens slices in a concentration-dependent manner (Fig. 4A). The IC_{50} of DP-7-AT for its inhibitory effect was approximately 10^{-8} M. DEγE, added to the superfusion medium 40 min prior to DP-7-AT at a concentration of 10^{-7} M, did not influence the half-maximal inhibition of the release of [^{3}H]DA caused by 10^{-8} M DP-7-AT (Fig. 4B). By itself, DEγE did not affect either the electrically stimulated release of [^{3}H]DA or the spontaneous efflux of radioactivity measured in the first collection period.

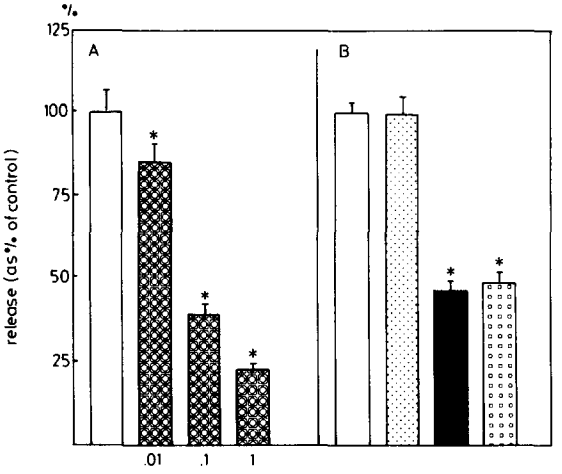

Fig. 4. DP-7-AT and the electrically evoked release of [^{3}H]DA from rat nucleus accumbens slices (A: graded concentrations of DP-7-AT; B: interaction between DEγE and DP-7-AT). Tissue was exposed once to electrical stimulation. DEγE, if present, was added to the medium at the start of the presuperfusion. DP-7-AT was added 40 min later, 20 min prior to stimulation (protocol 3). The values represent mean percentage ± S.E.M. of control. Conditions: (A) white column, control; circle in square, DP-7-AT (the molar concentration ($\times10^{-7}$ M) is indicated on the abscissa); (B) open column, control; dotted column, DEγE 10^{-7} M; black column, DP-7-AT 10^{-8} M; squares, DP-7-AT 10^{-8} M + DEγE 10^{-7} M. The number of observations was 6 (A) or 11–12 (B) per group. Results ANOVA: A: $F_{3,23} = 66.9$, $P < 0.001$; B: $F_{3,46} = 70.0$, $P < 0.001$. *Different from control ($P < 0.05$, Newman–Keuls).

DISCUSSION

Behavioural experiments have shown that the hypomotility induced by small doses of the DA receptor agonists apomorphine and bromocriptine can be blocked by DEγE at the level of the nucleus accumbens[8,24]. In recent experiments we have found that DEγE was also able to block the DP-7-AT-induced

hypomotility following injection into the nucleus accumbens (unpublished observations).

DP-7-AT is a newly developed drug displaying a high affinity towards the presynaptic DA receptor[4,9,19,29]. As shown in this study, both apomorphine and DP-7-AT inhibited the release of [^{3}H]DA from nucleus accumbens tissue in a concentration-dependent manner. Likewise, the selective D_2 DA receptor agonist LY 171555, the active enantiomer of the selective D_2 agonist LY 141865[6,17,18], was able to inhibit the release of [^{3}H]DA, suggesting that the DA receptor involved is a D_2 receptor. This suggestion is supported by the finding that the selective D_2 receptor antagonist (–)-sulpiride[7,17] was able to block the effects of 2×10^{-8} M apomorphine in a concentration-dependent manner. In this in vitro model of DA receptor-regulated DA release, the neuropeptide DEγE hardly influenced the inhibitory effects of DA agonists. This was tested under several conditions. We wish to emphasize the fact that the conditions chosen for electrical stimulation in the present study were very mild. It is well known that mild stimulation conditions are essential for the optimal detection of modulatory effects of drugs on neurotransmitter release[10,16]. DEγE, added to the superfusion medium at a concentration of 10^{-6} M simultaneously with the DA agonists, did not change the effects of the agonists, while added at a concentration of 10^{-7} M a small interaction with LY 171555 was observed. Focussing on this concentration of the peptide, experiments were performed in which 10^{-7} M DEγE was added to the superfusion medium 50 min prior to various concentrations of apomorphine. This experimental protocol closely resembled the protocol used in behavioural experiments by Van Ree et al.[24]. Also under these conditions, however, DEγE hardly influenced the effects of apomorphine. Finally, release experiments were performed under conditions in which only one electrical stimulation was used. This was done to prevent a possible interference of the first electrical stimulation with the mode of action of the drugs during the second stimulation. Again, under these experimental conditions the neuropeptide DEγE (10^{-7} M) did not alter the half-maximal inhibition of [^{3}H]DA release induced by 10^{-8} M DP-7-AT or 10^{-8} M apomorphine (data not shown). In none of the experiments was DEγE, by itself, able to affect the electrically stimulated release of [^{3}H]DA or the basal release of [^{3}H]DA as indicated by the spontaneous efflux of radioactivity measured in the first collection period.

Previously, other investigators have examined the effects of γ-type endorphins on [^{3}H]DA release in vitro. Schoemaker and Nickolson[15] found that DTγE did not affect basal DA release but depressed K^+-evoked release. Versteeg et al.[28] described a similar effect and demonstrated a relationship between DTγE's effect on DA release in vitro from nucleus accumbens slices and the pre-decapitation state of arousal of the rats. DTγE was found to exert a decreasing effect on K^+-induced release of [^{3}H]DA 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 prior to decapitation, this effect was absent, while an enhancing effect of DTγE became evident on basal DA efflux. As already mentioned, in our experiments, the neuropeptide DEγE did not affect either the electrically stimulated release or the basal release of [^{3}H]DA. Various reports have dealt with the presence or absence of effects of γ-type endorphins on dopaminergic activity in the nucleus accumbens of rats using other biochemical methods.

No effect was found for intracerebroventricularly administered DTγE on α-methyl-*p*-tyrosine (α-MPT)-induced disappearance of DA in the nucleus accumbens[26,27]. γ-Type endorphins did not displace [^{3}H]spiperone or [^{3}H]apomorphine from their binding sites in the nucleus accumbens in vitro[12,20], but effects have been found using in vivo binding[1]. Weinberger et al.[30], using conventional neuroleptic tests as well as synaptosomal DA formation, found markedly different response profiles between DTγE and haloperidol. Obviously it seems difficult to convincingly demonstrate the postulated antidopaminergic activity of these neuropeptides in biochemical experiments.

From the present data we conclude that DEγE does not function in the nucleus accumbens as an antagonist for the DA receptor that mediates the inhibition of the release of previously accumulated [^{3}H]DA, that has the characteristics of a D_2 DA receptor and that is presumably located on the nerve terminals. It, therefore, seems unlikely that the interaction observed in behavioural experiments between DA receptor agonists and DEγE occurs at the level

of the DA receptor mediating DA release, although it may be that this interaction cannot be detected by using this in vitro superfusion technique. Thus, analysis of the influence of DEγE on the apomorphine-induced inhibition of the release of endogenous DA in vivo may contribute to elucidate the interaction between DEγE and dopaminergic systems in the nucleus accumbens. It is, however, possible that the behavioural antagonism between apomorphine and DEγE occurs at another level, e.g. has the character of a post-DA receptor-mediated event via indirect presynaptic mechanisms.

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