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Glutamate-evoked release of endogenous brain dopamine: inhibition by an excitatory amino acid antagonist and an enkephalin analogue

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1 The present study examined the effect of a selective δ-opioid receptor agonist [D-Ala^2-D-Leu^5] enkephalin (DADL) on the spontaneous and the L-glutamic acid (L-Glu)-evoked release of endogenous dopamine from superfused slices of rat caudate-putamen. The amount of dopamine in slice superfusates was measured by a sensitive method employing high-performance liquid chromatography with electrochemical detection (h.p.l.c.-e.d.) after a two-step separation procedure.

2 The spontaneous release of endogenous dopamine was (a) partially dependent on Ca^{2+}, (b) enhanced in Mg^{2+}-free superfusion medium, (c) partially reduced by tetrodotoxin (TTX, 0.3 μM), (d) partially reduced by the putative excitatory amino acid receptor antagonist DL-2-amino-7-phosphono-heptanoic acid (DL-APH, 1 mM), and (f) increased 10 fold by the dopamine uptake blocker, nomifensine (10 μM). DADL (5 and 50 nM) did not significantly affect spontaneous dopamine release.

3 L-Glu (0.1–10 mM) produced a concentration-dependent release of endogenous dopamine from slices of caudate-putamen. This effect was (a) Ca^{2+}-dependent, (b) strongly inhibited by 1.2 mM Mg^{2+}, (c) attenuated by DL-APH (1 mM), (d) attenuated by TTX (0.3 μM), and (c) enhanced by nomifensine (10 μM). In the presence of nomifensine DADL (50 nM) reduced significantly the L-Glu-evoked release of endogenous dopamine by 20%. The inhibitory effect of DADL was blocked by 10 μM naloxone.

4 These results indicate tht L-Glu stimulates the Ca^{2+}-dependent release of endogenous dopamine in the caudate-putamen by activation of N-methy-D-aspartate-type of excitatory amino acid receptors. This release can be selectively modified by the δ-opioid agonist DADL in a naloxone-sensitive manner.

Introduction

Numerous studies have indicated that opioid agonists, including enkephalins, modify dopaminergic neurotransmission in the rat striatum. Injection of the enkephalin analogue (N-Me-Tyr-D-Ser-Gly-N-Me-Phe-D-Ser-NH_2) significantly reduces the amphetamine-evoked stereotyped behaviour and striatal dopamine release *in vivo*, as measured by chronoamperometry (Broderick *et al.*, 1983). Local administration of enkephalins into the rat striatum induces changes in dopamine synthesis and metabolism that are consistent with alterations in the *in vivo* release of dopamine in this region (Wood, 1983). These latter effects of opioids are thought to be mediated by a population of opioid receptors localized on the terminals of the dopaminergic nigrostriatal afferents since the destruction of striatal cell bodies by kainic acid, or the removal of corticostriatal projections by decortication, fails to modify such effects (Pollard *et al.*, 1978). Autoradiographic studies have suggested that the opioid binding sites associated with the nigrostriatal terminals have a diffuse pattern of distribution in the rat striatum characteristic of δ-type opioid receptors (Goodman *et al.*, 1980). The possibility that opioids influence presynaptic dopamine function is supported by some *in vitro* studies showing that opioids influence [^{3}H]-dopamine release from striatal slices. However, the results of *in vitro* [^{3}H]-dopamine release studies are controversial as they demonstrate that opioid peptides decrease (Loh *et al.*, 1976; Subramanian *et al.*, 1977; Mulder *et al.*, 1984), increase (Lubetzki *et al.*, 1982), or do not influence this release (Arbilla & Langer, 1978). The reasons for these discrepancies are unclear but are possibly related to methodological differences. To date little attention has focused on the effects of opioids on the release of endogenous dopamine *in vitro*.

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Considering that the release of dopamine in the striatum is influenced by other neurotransmitters that are either intrinsic or project to this region, it is conceivable that opioids influence the presynaptic release of dopamine by modifying the function of one or more of these neurotransmitters. In this regard L-glutamic acid (L-Glu), an excitatory amino acid, and a candidate for the transmitter in the corticostriatal tract, is of special interest. Previous studies have shown that L-Glu increases the release of [^{3}H]-dopamine from striatal slices in a manner that is consistent with activation of presynaptic glutamate receptors on the afferents of the nigrostriatal pathway (Roberts & Anderson, 1979; Marien *et al.*, 1983). Opioids have been found to depress L-Glu-evoked neuronal excitation in electrophysiological experiments (Zieglgansberger & Bayerl, 1976; Barker *et al.*, 1978) and to inhibit the L-Glu-evoked release of ^{3}H-purines or [^{3}H]-dopamine from the rat cerebral cortex and striatum respectively (Jhamandas & Dumbrille, 1980; Marien *et al.*, 1983). These studies therefore suggest a possible modulation of L-Glu-induced responses by opioids in the striatum.

In this study we have investigated the action of L-Glu on the release of endogenous dopamine from the rat caudate-putamen *in vitro*, and have examined the effects of a glutamate receptor antagonist and an enkephalin analogue on this release. The measurement of endogenous dopamine has been performed using high performance liquid chromatography with electrochemical detection (h.p.l.c.-e.d.) after a two-step separation of dopamine.

Methods

Tissue preparation and superfusion

Male Sprague-Dawley rats weighing between 180 g and 250 g were used in all experiments. Animals were housed at a constant temperature (20°C) in a programmed light environment (12 h light, 12 h dark) with free access to food and water. Animals were decapitated (between 10 h 00 min and 14 h 00 min), the brains quickly removed and rinsed briefly with ice-cold modified Krebs-Henseleit bicarbonate medium. Left and right caudate-putamen were removed and cut sagitally into 300 μm-thick slices with a McIlwain tissue chopper. Equal portions of left and right caudate-putamen tissue were transferred to two 20 ml beakers containing 3 ml medium for slice dispersion. Unless otherwise specified, modified Krebs-Henseleit bicarbonate medium was of the following composition (mM): NaCl 118, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.3, $NaHCO_3$ 25, glucose 10. Disodium ethylenediaminetetraacetate (Na_2EDTA, 0.045 mM) and sodium metabisulphite ($Na_2S_2O_5$, 0.53 mM) were included to prevent the oxidation of released dopamine. The medium was maintained at 37°C in a water bath and equilibrated to pH 7.2–7.4 by constant gassing with 95% O_2 – 5% CO_2.

Superfusion experiments

The two beakers of dispersed caudate-putamen slices were emptied into two separate superfusion chambers (5 ml) maintained at 37°C. Slices from a second rat brain were then prepared and distributed to the two superfusion chambers as described above. In this way, each chamber contained equal portions of left and right caudate-putamen slices from both brains (50–70 mg total wet weight per chamber). The slices were superfused with prewarmed gassed perfusion medium at a flow rate of 0.5 ml min^{-1}. During the superfusion experiment, serial 10 min samples of superfusate were collected from the bottom end (outflow) of the chambers into cold centrifuge tubes containing 200 μl of HCl (1 M) and 40 μl of freshly-prepared $Na_2S_2O_5$ (0.26 M).

At selected times during a 70 min superfusion experiment, slices were exposed to the drugs or ionic conditions under investigation by transferring the chamber inflow tubings to reservoirs containing superfusion medium of appropriate composition. In spontaneous dopamine release experiments, drugs were present in the medium for 30 min, from 40 to 70 min of superfusion time. In the experiments in which L-glutamic acid (L-Glu) was used to stimulate dopamine release, superfusion medium containing L-Glu was applied to the caudate-putamen slices for 5 min, from 40 to 45 min of superfusion time. When drugs were tested against the L-Glu-evoked dopamine release, they were usually present in the medium from 0 or 20 min to 70 min of superfusion. In each paired release experiment, the slice preparation in one chamber was randomly assigned for 'control' observations and the other for 'test' observations. Immediately at the end of the superfusion experiment (70 min), the slices were removed from the chambers, blotted and weighed. Acidified superfusate samples (final pH 1.5–1.79 were stored at 0–4°C before the separation and estimation of dopamine.

Separation and estimation of dopamine

Dopamine was extracted from superfusate samples by the alumina adsorption and cation-exchange techniques as described by Hamlet *et al.* (1981). In brief, the catechols adsorbed onto alumina were eluted by shaking for 10 min with 6 ml of 0.05 M perchloric acid. Following centrifugation, the perchloric acid supernatants were transferred to 20 ml polyethylene vials on ice containing 100 μl each of freshly-prepared Na_2EDTA (0.15 M) and ascorbic acid (5.68 M). The pH

of this mixture was carefully adjusted to a value between 6.0–6.2 using 0.1 M Na_2CO_3. The samples were then applied to small columns (bed height 30 mm, diameter 3 mm) of Biorex 70 cation-exchange resin (100–200 mesh, BioRad Laboratories, Richmond, CA). The column resin was washed with 5 ml of glass-distilled water and dopamine eluted with 1 ml of 0.67 M boric acid.

The amount of dopamine in aliquots of the boric acid eluates was measured by h.p.l.c.-e.d. using a Model M-45 solvent (Waters Scientific Ltd, Mississauga, Ontario) and a μBondapak (10 μm, C_{18}, 4.6 × 300 mm; Waters Scientific) or Biophase ODS column (5 μm, C_{18}, 4.6 × 250 mm; Bioanalytical Systems Inc., West Lafayette, IN) and an electrochemical detector (Bioanalytical Systems Inc.). The oxidation potential at the working electrode was set by the controller at 0.65 V against a Ag/AgCl reference electrode. Detector output was recorded on a strip chart recorder (Houston Instruments). The isocratic solvent system consisted of 0.07 M NaH_2PO_4 (pH 4.8) containing 0.4 mM sodium heptanesulphonate, 0.2 mM), Na_2EDTA and 7.5% (v/v) methanol, and was delivered at a flow rate of 1.5 ml min^{-1} with a corresponding backpressure of 2800–3300 p.s.i. Under these chromatographic conditions, the retention time of dopamine was usually 6.8 min.

Expression of release data and statistical analysis

The rate of dopamine release into each superfusate sample is expressed as the total amount of dopamine measured in the respective 10 min (5 ml) sample per 100 mg of superfused tissue. Unless indicated otherwise, observations from slices in 'control' superfusion chambers were compared with observations from slices in 'test' chambers using Student's two-tailed paired t test. Differences between observed values were considered significant if $P < 0.05$. Unless indicated otherwise, results are presented as means ± s.e.mean.

Guinea-pig ileum myenteric plexus longitudinal muscle preparation

This tissue preparation was used to investigate the opioid agonist or antagonist activity of some of the drugs tested in the dopamine release experiments.The tissue was prepared as described in detail in an earlier publication (Sawynok & Jhamandas, 1976).

Drugs, reagents and solutions

The compounds used in this study were obtained from the following sources: [D-Ala^2-D-Leu^5] enkephalin (DADL), dopamine hydrochloride, L-glutamic acid (Sigma Chemical Co.); naloxone hydrochloride (Endo Laboratories Inc.); nomifensine mealeate (Hoechst Canada Inc.); DL-2-amino-7-phosphonoheptanoic acid (DL-APH) (Tocris Chemicals); other chemicals were reagent or h.p.l.c. grade. Glass-distilled water was used for preparing all solutions. DADL was dissolved in 0.01 M acetic acid; other drugs were dissolved in water or perfusion medium. These solutions and dilutions thereof were added to superfusion medium reservoirs (silanized glass) in volumes not exceeding 5 ml l^{-1}. The final pH of superfusion medium containing drugs was 7.2–7.45. In control conditions, appropriate volumes of solvent were added to the superfusion medium reservoirs. Stock solutions of dopamine (1 mg ml^{-1}) were made up in 0.01 M HCl containing 0.1 mM Na_2EDTA and stored in 100 μl aliquots under N_2 in 500 μl size polypropylene microcentrifuge tubes at −70°C. Working solutions of dopamine for preparation of external standards in Krebs-Henseleit medium were prepared by serial dilution of 0.01 M HCl. For chromatograph calibration, working solutions of dopamine were prepared by serial dilution of 0.67 M boric acid.

Results

Under the separation and chromatographic conditions that were employed in this study, the minimum detectable quantity of dopamine was 3–7 pg in 50 μl injected onto the column. The within-day reproducibility of the detector response (peak heights generated) was tested through replicate injections of authentic dopamine standards (0.10–6.0 ng dopamine ml^{-1} 0.67 M boric acid). The coefficient of variation of the peak heights generated was less than 2% for dopamine standards which were 0.2 ng ml^{-1} or greater, and was less than 8% for standards under 0.2 ng ml^{-1} (6 injections per standard). The detector response was linear over the range of dopamine standards analysed (coefficient of determination $\geq$ 0.997). The average recovery of dopamine was 74%. The limit of sensitivity of the dopamine assay in the release experiments was 0.140 ng dopamine 100 mg^{-1} tissue per 5 ml (10 min) superfusate sample.

Spontaneous and L-glutamic acid-evoked release of endogenous dopamine from caudate-putamen slices

Dopamine was consistently detected in the 10 min superfusate samples over the entire time course of the 70 min superfusion experiments. As illustrated in Figure 1, the spontaneous release of dopamine stabilized to an average basal rate of 0.329 ng 100 mg^{-1} tissue 10 min^{-1} after the first 10 min collection period ('washout'). In some experiments, the level of dopamine remaining in the slices at the end of superfusion was determined to calculate the percen-

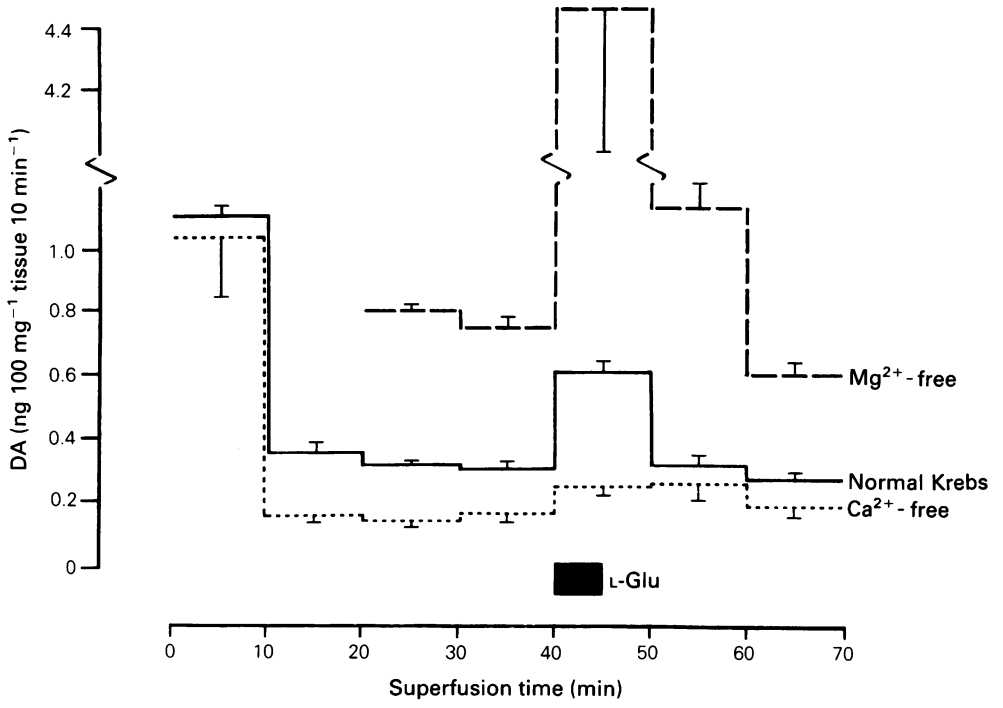

Figure 1 Spontaneous and L-glutamic acid (L-Glu, 1 mM)-evoked release of endogenous dopamine (DA) from superfused slices of rat caudate-putamen in normal, Ca^{2+}-free and Mg^{2+}-free solution. Normal perfusion medium contained 1.3 mM Ca^{2+} and 1.2 mM Mg^{2+}. The three ionic conditions used were present from the start of superfusion. The solid bar indicates the period of exposure to medium containing 1 mM L-Glu. Ordinate scale: rate of dopamine released from caudate-putamen slices, expressed as the amount of dopamine collected in a 10 min superfusion interval per 100 mg of superfused slices. Abscissa scale: time in min after the start of superfusion. Each value represents the mean of 4 experiments for each medium used and vertical lines show s.e.mean. Note that the ordinate scale is compressed to accommodate the Mg^{2+}-free data. All release rate values in both the Mg^{2+}-free and Ca^{2+}-free plots are significantly different ($P < 0.05$) from the corresponding values in the normal medium, as determined by Student's *t* tests.

tage of tissue dopamine released from the slices. The average rate of spontaneous dopamine release was 0.063% of total tissue content per 10 min.

The dependency of dopamine release on Ca^{2+} was examined by omitting the Ca^{2+} from the superfusion medium. Under these conditions, the spontaneous and the L-Glu-evoked release of dopamine was significantly reduced to 45% and 42% of control release respectively, as illustrated in Figure 1.

In a previous study, we demonstrated that low concentrations of Mg^{2+} antagonized the L-Glu-evoked overflow of ^3H from caudate-putamen slices pre-incubated with [^{3}H]-dopamine (Marien *et al.*, 1983). To evaluate the effect of Mg^{2+} on the release of endogenous dopamine, experiments were performed under normal and under Mg^{2+}-free superfusion conditions. As shown in Figure 1, in the Mg^{2+}-free solution the spontaneous and the L-Glu-evoked release of dopamine was 0.774 and 4.459 ng 100 mg^{-1} tissue 10 min^{-1} respectively. This represented a 246% increase in the spontaneous release and a 730% increase in the L-Glu-evoked release, when compared to release in normal perfusion medium. Thus, as was evident in the previous [^{3}H]-dopamine release study, Mg^{2+} markedly inhibited the L-Glu-evoked release of dopamine. In view of the inhibitory effect of this cation, Mg^{2+}-free perfusion medium was employed in all subsequent superfusion experiments, unless specified otherwise.

The ability of L-Glu to stimulate dopamine release from caudate-putamen slices was concentration-dependent, as illustrated in Figure 2. The apparent EC_{50} value for L-Glu was estimated to be approximately 2.0 mM. In subsequent experiments in which the effect of drugs on the evoked release of endogenous dopamine was examined, a submaximal concentration

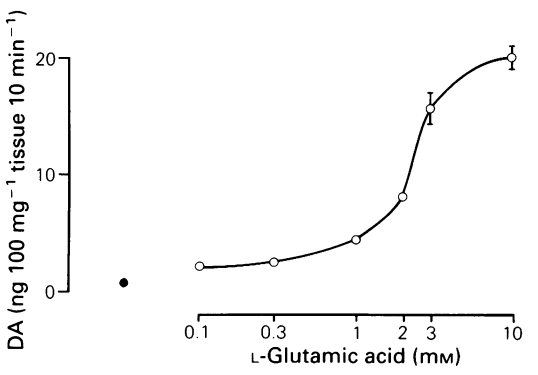

Figure 2 Concentration-dependent effect of L-glutamic acid of (L-Glu) on the release of endogenous dopamine (DA) from superfused caudate-putamen slices. Slices were superfused with Mg^{2+}-free medium in experiments similar to those illustrated in Figure 3. Slices were exposed to L-Glu in the concentration range 0.1–10.0 mM. (○) Represent the amount of dopamine released per 100 mg tissue during the superfusion time of 40–50 min. (●) Spontaneous (pre-glutamate) release of dopamine during the superfusion time of 30–40 min. Values are mean of 3–22 experiments, s.e.mean was less than 10% of the value indicated by each symbol.

of L-Glu (1 mM) was used to induce release.

Effect of DL-2-amino-7-phosphonoheptanoic acid and tetrodotoxin

To assess whether glutamate receptors were involved in the action of L-Glu on dopamine release, experiments were performed in the presence of DL-APH, a putative glutamate receptor antagonist (Perkins *et al.*, 1982). In the presence of 1 mM DL-APH, the spontaneous and the L-Glu-evoked release of dopamine were reduced to 61% and 50% respectively of the release occurring in the absence of this agent (see Table 1).

To determine if the ability of L-Glu to stimulate dopamine release from caudate-putamen slices was dependent on the generation of propagated nerve activity, experiments were conducted in the presence of tetrodotoxin (TTX, 0.3 μM) which is known to inhibit the generation and conduction of action potentials by blocking Na^+ channels. The results are shown in Table 1. TTX reduced the spontaneous release of dopamine to 40% of control levels. In the presence of TTX the acutal amount of dopamine released by L-Glu was reduced when compared with release in the absence of TTX.

Effect of DADL on dopamine release

The effect of DADL on the spontaneous release of dopamine was examined by exposing caudate-putamen slices to normal perfusion medium containing the enkephalin from 40 to 70 min of superfusion. DADL (5 and 50 nM) had no significant action on the spontaneous release of dopamine (data not shown). To investigate the effect of DADL on L-Glu-evoked dopamine release, caudate-putamen slices were exposed to Mg^{2+}-free perfusion containing DADL from 20 to 70 min of superfusion, and were stimulated at 40–45 min by medium containing 1 mM L-Glu. Results of these experiments are shown in Figure 3. DADL (50 nM) had no significant effect on either the pre-glutamate or the glutamate-evoked release of dopamine when compared to the control effect. A similar lack of effect on the spontaneous and evoked release was observed when 5 nM DADL was tested (data not shown). To determine that the lack of effect

Table 1 Effects of DL-2-amino-7-phosphonoheptanoic acid (DL-APH, 1 mM) and tetrodotoxin (TTX, 0.3 μM) on the spontaneous and the L-glutamic acid (L-Glu, 1 mM)-evoked release of endogenous dopamine from superfused caudate-putamen slices

Drug present in superfusion medium	*Dopamine release* (ng 100 mg^{-1} 10 min^{-1}) *Spontaneous*	*L-Glu-evoked*
Control	1.057 ± 0.081 (4)	6.393 ± 0.0863 (4)
DL-APH	0.650 ± 0.082 (4)* [61%]	3.215 ± 0.646 (4)* [50%]
Control	1.063 ± 0.106 (4)	5.737 ± 0.610 (4)
TTX	0.426 ± 0.051 (4)* [40%]	2.887 ± 0.368 (4)* [50%]

Caudate-putamen slices were superfused with Mg^{2+}-free perfusion medium. DL-APH (1 mM) and TTX (0.3 μM were present in the medium from the start of superfusion. Dopamine release values are mean ± s.e.mean and number of experiments are shown in parentheses. Spontaneous release represents release during 30–40 min superfusion time and L-Glu-evoked release represents that occurring during 40–50 min superfusion time. Tissues were exposed to 1 mM L-Glu at 40–45 min. Square brackets indicate release values expressed as % control. Significance of the drug effect, indicated by *($P < 0.05$), was assessed by comparing the release in control and drug experiments by means of Student's paired *t* test.

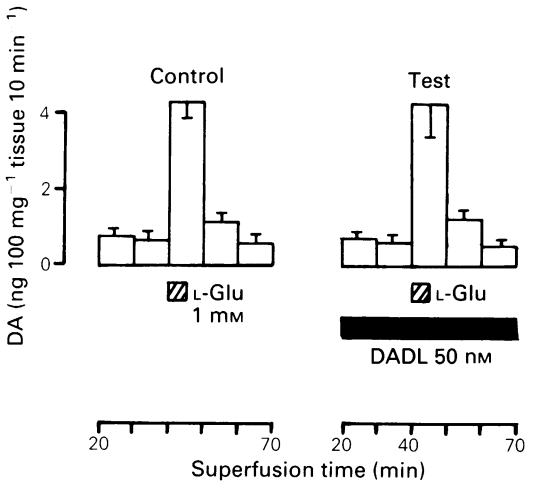

Figure 3 The effect of [D-Ala²-D-Leu⁵] enkephalin (DADL) on L-glutamic acid (L-Glu)-evoked release of endogenous dopamine (DA) from superfused caudate-putamen slices in the absence of a dopamine uptake blocker. The L-Glu-evoked release experiments were performed in Mg^{2+}-free medium. The solid bar indicates the period of exposure to DADL. The hatched bar indicates the 5 min period of exposure to L-Glu 'Control' and 'test' experiments were performed at the same time. Ordinate scale: rate of dopamine released from caudate-putamen slices into the superfusion medium. Abscissa scales: time in min after the start of superfusion. Each column represents the mean of 4 experiments and vertical lines show s.e.mean.

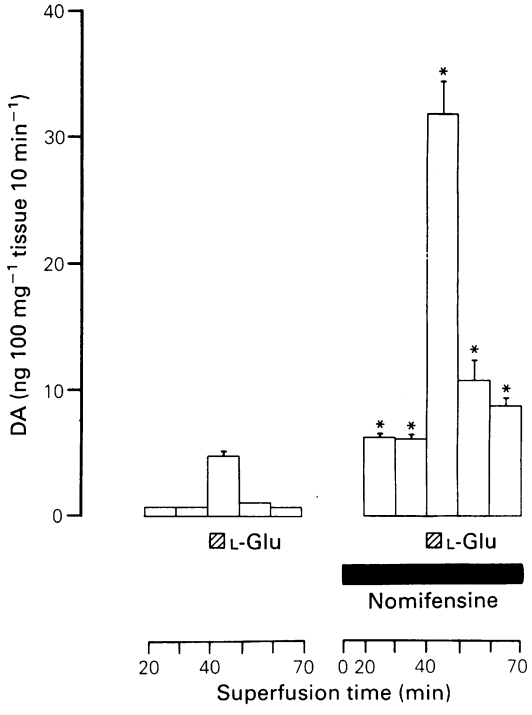

Figure 4 The effect of nomifensine (10 μM) on the L-glutamic acid (L-Glu)-evoked release of endogenous dopamine (DA) from superfused caudate-putamen slices. Experiments were performed in Mg^{2+}-free medium. The hatched bar indicates the 5 min period of exposure to L-Glu (1 mM). The solid bar indicates the period of exposure to nomifensine (10 μM). Ordinate scale: rate of dopamine released from caudate-putamen slices into the superfusion medium. Abscissa scales: time in min after the start of superfusion. Each column represents the mean of 21 experiments (L-Glu alone) or 19 experiments (L-Glu + nomifensine); s.e.mean is indicated by a vertical line except where it is too small to incorporate into the figure. $*P < 0.001$ when compared to release values in absence of nomifensine by Student's unpaired t test.

of the enkephalin was not due to a lack of opioid activity of the stock drug, the biological activity of DADL was evaluated in the field-stimulated guinea-pig ileum myenteric plexus longitudinal muscle preparation. DADL reduced the tissue contractile responses and its inhibitory effect was almost completely reversed by 0.5 μM naloxone (not shown).

Effect of DADL on dopamine release in the presence of nomifensine

As certain opioids have been shown previously to influence dopamine uptake (George & Van Loon, 1982), it was of interest to examine the effect of the opioid peptide DADL in release experiments in which the dopamine reuptake process was blocked. Such experiments were therefore conducted in the presence of the selective dopamine uptake blocker nomifensine. Nomifensine is a tetrahydroisoquinoline derivative and some drugs in this class of compounds have been found to interact with opioid receptors (Hamilton *et al.*, 1979). However, when tested in the guinea-pig ileum myenteric plexus longitudinal muscle preparation, nomifensine, at concentrations up to 10^{-4} M, did not appear to have either opioid agonist or antagonist activity (not shown).

Before performing release experiments with opioids in the presence of nomifensine, its action on dopamine release was examined in separate experiments. Figure 4 shows that nomifensine, at a concentration (10 μM) which is known to eliminate virutually all dopamine uptake (Raiteri *et al.*, 1978), caused a 10 fold increase in the spontaneous release of dopamine. This effect was observed in the first 10 min of exposure to nomifensine. The absolute quantity of dopamine released from caudate-putamen slices as a result of

stimulation by L-Glu was also increased in the presence of nomifensine. However, the relative increase in the release of dopamine produced by L-Glu was not significantly affected in the presence of nomifensine. In the absence of nomifensine, 1 mM L-Glu increased the rate of dopamine release by 573 ± 33% ($n = 21$) while in the presence of this agent, it increased the release by 520 ± 20% ($n = 19$).

The effect of DADL (5 and 50 nM) on both the spontaneous and the L-Glu-evoked release of dopamine was examined in the presence of 10 μM nomifensine. The results of spontaneous release tests are represented in Table 2 and those of evoked release are shown in Figure 5. DADL (5 or 50 nM) did not significantly alter the spontaneous release of dopamine from caudate-putamen slices in the presence of nomifensine (Table 2). In contrast, DADL (50 nM) significantly reduced the L-Glu-evoked release of dopamine by about 20%. The opioid antagonist naloxone (10 μM), which by itself had no significant effect on dopamine release, blocked the inhibitory effect of 50 nM DADL (Figure 5). A lower concentration of DADL (5 nM) was also tested, but this did not modify the glutamate-evoked release.

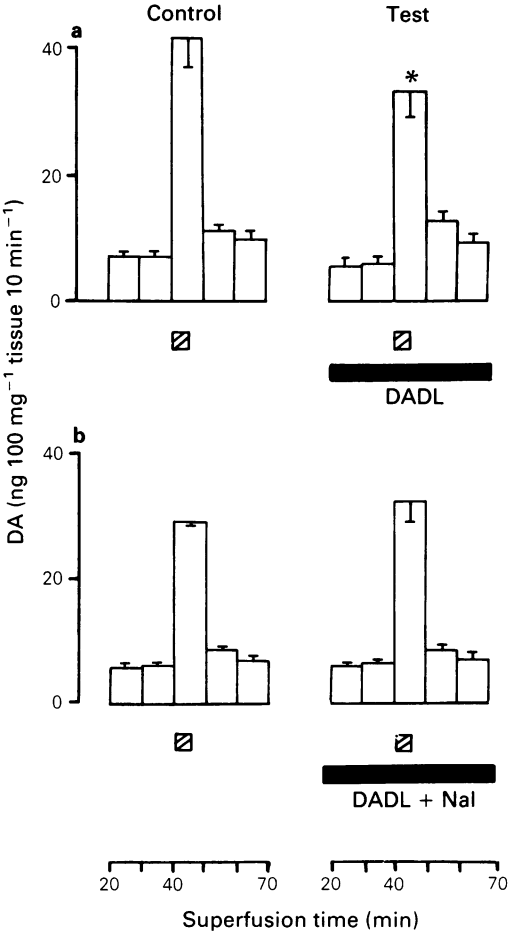

Figure 5 The effect of [D-Ala²-D-Leu⁵] enkephalin (DADL) on L-glutamic acid (L-Glu)-evoked release of endogenous dopamine (DA) from superfused caudate-putamen slices in the presence of an uptake blocker, nomifensine (10 μM). The release experiments were performed in Mg^{2+}-free medium. The solid bar indicates period of exposure to (a) DADL or (b) DADL and naloxone (Nal). The hatched bar indicates 5 min exposure to L-Glu. 'Control' and 'test' experiments were performed at the same time. Ordinate scales: rate of dopamine released from caudate-putamen slices into the superfusion medium Abscissa scale: time in min after the start of superfusion. Each column represents the mean of 3–5 experiments and vertical lines show s.e.mean. $*P < 0.05$ when compared to corresponding control value by Student's unpaired t test.

Discussion

L-Glutamate action on dopamine release

L-Glu stimulated the release of endogenous dopamine from superfused slices of the rat caudate-putamen in a dose- and Ca^{2+}-dependent manner. The observed effect of L-Glu appears to be mediated by the N-methyl-D-aspartate (NMDA)-type excitatory amino acid receptors as it was reduced by Mg^{2+} or the NMDA-receptor antagonist DL-APH. The high sensitivity of glutamate to inhibition by Mg^{2+} has also been observed in release studies on striatal [^{3}H]-dopamine (Roberts & Anderson, 1979; Marien *et al.*, 1983). It is unlikely that the action of DL-APH is non-specific as structurally-related compounds (aminophosphonovaleric and aminophosphonobutyric acids) at similar concentrations have been found to be devoid of non-specific effects (Evans *et al.*, 1982). Although the concentration of DL-APH used here was rather high, the glutamate effect on dopamine release was not completely blocked. This may be due to the possibility that the L-isomer present in the racemic mixture, known to have a weak depolarizing action (Perkins *et al.*, 1982), opposed the antagonistic action of the D-isomer. Another hypothesis for the incomplete antagonism is that glutamate action on dopamine release is partly mediated through other excitatory amino acid receptor sites, such as the Mg^{2+}-insensitive 'quisqualate' or 'kainate' sites. However, the powerful inhibitory action of Mg^{2+} against glutamate points to a predominant role of NMDA receptors. A reduction in the spontaneous release of dopamine by the antagonist suggests that this release may be under a

Table 2 Effect of D-Ala²-D-Leu⁵] enkephalin (DADL) on the spontaneous release of endogenous dopamine from the superfused caudate-putamen slices in the presence of nomifensine

Drug (nM) present in superfusion medium	*Dopamine release (ng 100 mg^{-1} 10 min^{-1})*				
	20–30	*30–40*	*40–50*	*50–60*	*60–70*
Control (6)	3.631 ± 0.222	4.073 ± 0.245	5.021 ± 0.410	6.236 ± 0.591	7.922 ± 0.705
DADL, 5 (6)	4.221 ± 0.382	4.857 ± 0.542	5.953 ± 0.945	7.448 ± 1.076	9.507 ± 1.376
Control (3)	3.134 ± 0.246	3.439 ± 0.241	3.774 ± 0.295	4.601 ± 0.459	5.884 ± 0.685
DADL, 50 (3)	3.078 ± 0.474	3.526 ± 0.724	3.595 ± 0.690	4.618 ± 1.024	5.602 ± 1.186

Caudate-putamen slices were superfused with normal superfusion medium containing 10 μM nomifensine. DADL was present in the medium from 40–70 min superfusion time. Release values shown are mean ± s.e.mean and number of experiments are shown in parentheses. Release of dopamine in the absence of DADL (control) was compared to release in the presence of dug by means of Students's paired *t* test to assess significance of the drug effect.

tonic excitatory influence of endogenous glutamate in the caudate-putamen slice preparation.

TTX, used here at a concentration known to abolish the release of [³H]-dopamine induced by electrical stimulation (Starke *et al.*, 1978), but not by L-Glu, reduced the baseline and the L-Glu-evoked release of endogenous dopamine. The effect of TTX on baseline release suggests that a part of this release is due to propagation of action potentials in the caudate-putamen slice preparation. The persistence of L-Glu-evoked release in the presence of TTX suggests that the amino acid may be exerting a direct presynaptic action on the dopamine nerve terminal in the caudate-putamen region. This conclusion is supported by the observation of Roberts *et al.* (1982) that glutamate receptor binding in the rat decreases by 40% after a nigral lesion. However, De Belleroche & Bradford (1980) showed that L-Glu does not influence the release of [¹⁴C]-dopamine from striatal synaptosomes, a finding that is in apparent conflict with the above conclusion. The lack of effect of L-Glu in the synaptosomal experiments can be explained by the presence of magnesium ions in the incubation medium.

Action of nomifensine on dopamine release

Nomifensine, an inhibitor of dopamine uptake, in the present study caused an immediate 10 fold increase in dopamine release. Previous studies have shown that unlike amphetamine and benztropine, two agents known to inhibit dopamine uptake, nomifensine had no stimulatory action on the release of [³H]-dopamine from rapidly superfused synaptosomes (Raiteri *et al.*, 1978). However, this drug clearly increased the spontaneous release of endogenous dopamine. This effect may be due to the inhibition of an ongoing re-uptake of dopamine under the conditions used in this study. It has previously been shown that 10 μM nomifensine increases dopamine synthesis in rat striatal synaptosomes (Maura & Raiteri, 1982). The increase in dopamine release over time observed here may represent release of the transmitter from a newly-synthesized pool that gradually increases as the intraterminal levels of recaptured dopamine decrease.

Effect of DADL on endogenous dopamine release

In the absence of the dopamine uptake blocker nomifensine, DADL had no observable effect on the spontaneous or the L-Glu-evoked release of endogenous dopamine. The present findings are in direct contrast to the finding that concentrations of DADL employed in the present study enhance the spontaneous efflux of [³H]-dopamine from slices of rat striatum superfused with a medium containing [³H]-tyrosine (Lubetzki *et al.*, 1982). The reasons for this discrepancy are unclear, but they may be related to the basic methodological difference between the studies. In the presence of nomifensine, 50 nM DADL was still without effect on the spontaneous release of dopamine, but it reduced the glutamate-evoked release by about 20% in a naloxone-sensitive manner. At the dose used here naloxone itself had no action on dopamine release. This observation is in accord with the finding of Mulder *et al.* (1984) that DADL reduced the K^+-evoked release of [³H]-dopamine by about 15%. DADL has been shown to interact preferentially with δ-receptors in isolated tissues (Wuster *et al.*, 1978). Furthermore, at 50 nM, the concentration of DADL that was effective in modifying L-Glu-evoked dopamine release, the peptide had little or no effect in reducing the twitch response of the field stimulated guinea-pig ileum myenteric plexus longitudinal muscle preparation, a tissue which contains predominantly μ-receptors. Thus, the modest DADL-induced depression of dopamine release in the present study appears to be mediated by an activation of δ-receptors. The site of the interaction between DADL and L-Glu in the

caudate-putamen is most likely to be the dopaminergic nerve terminal, since both glutamate (Roberts *et al.*, 1982) and δ-opioid binding sites (Goodman *et al.*, 1980) exist on this terminal. However, the type of opioid receptor involved in mediating the enkephalin action needs to be investigated further in future experiments by the use of more selective opioid agonists and antagonists.

It is not clear why the inhibitory effect of DADL was evident only in the presence of nomifensine. It is possible that when the re-uptake of dopamine is inhibited, L-Glu releases dopamine from a neuronal pool (which may contain newly synthesized transmitter) that is more sensitive to the inhibitory action of the peptide. Since this effect of DADL was observed only under specific conditions, the exact physiological relevance of the receptors activated by this peptide remains to be determined.

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