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ACUTE EFFECTS OF D-1 AND D-2 DOPAMINE RECEPTOR AGONIST AND ANTAGONIST DRUGS ON BASAL GANGLIA [MET^5]- AND [LEU^5]-ENKEPHALIN AND NEUROTENSIN CONTENT IN THE RAT

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Abstract—The effects of acute systemic injection of the D-1 agonist SKF 38393 (2.5–20 mg/kg) or the D-1 antagonist SCH 23390 (0.25–2.0 mg/kg), and of the D-2 agonist quinpirole (0.12–1.0 mg/kg) or the D-2 antagonist sulpiride (25–100 mg/kg) on the neuropeptide content of rat basal ganglia were investigated. In striatum, the [Met^5]- and [Leu^5]-enkephalin content was unaffected by administration of SKF 38393 or SCH 23390. Quinpirole had no effect on [Met^5]- and [Leu^5]-enkephalin levels but sulpiride produced an increase in both [Met^5]- and (Leu^5]-enkephalin content. In the nucleus accumbens, SKF 38393 decreased and SCH 23390 increased [Met^5]- and [Leu^5]-enkephalin levels. Quinpirole decreased [Met^5]- and [Leu^5]-enkephalin levels, while sulpiride decreased [Leu^5]-enkephalin levels alone. The content of [Leu^5]- but not [Met^5]-enkephalin levels in the substantia nigra was increased by administration of SKF 38393, and decreased by SCH 23390. Quinpirole and sulpiride were without effect on the [Met^5]- or [Leu^5]-enkephalin content of substantia nigra. Neurotensin levels in striatum were increased by administration of SKF 38393 and decreased by SCH 23390. Similarly, quinpirole decreased the neurotensin content while sulpiride caused an increase. In the nucleus accumbens, the neurotensin content was not affected by administration of SKF 38393 but increased by SCH 23390. Neither quinpirole nor sulpiride altered neurotensin levels in the nucleus accumbens. Neurotensin levels in substantia nigra were unaffected by the administration of SKF 38393 and SCH 23390, or by quinpirole and sulpiride. These results indicate that acute administration of D-1 and D-2 agonist and antagonist drugs can alter the levels of [Met^5]- and [Leu^5]-enkephalin and neurotensin in basal ganglia. However, there are marked differences between brain regions in the regulation of peptide levels by acute D-1 and D-2 receptor occupation.

The subchronic administration of drugs that alter brain dopamine function can effect the basal ganglia neuropeptide content. Thus, treatment with neuroleptics, which are central dopamine receptor antagonists, for several weeks increased [Met^5]-enkephalin levels in striatum and nucleus accumbens, and those of [Leu^5]-enkephalin in striatum and substantia nigra [1–3]. Similar results have also been obtained employing continuous neuroleptic administration over 18 months [4]. In contrast, long term treatment with dopamine agonist drugs decreased [Met^5]-, but not [Leu^5]-enkephalin levels in striatum [4]. Acute administration of typical neuroleptics is reported not to result in enkephalin level alterations. However, a single injection of the atypical neuroleptic sulpiride increased [Met^5]-enkephalin content in striatum, but decreased it in substantia nigra and nucleus accumbens [5]. [Leu^5]-enkephalin levels in this study were also decreased in nucleus accumbens but not elsewhere. These results suggested that sulpiride effects could be due to its selective D-2 dopamine receptor antagonist action.

Similarly to the enkephalins, the neurotensin content of the striatum and nucleus accumbens was enhanced after subchronic [6–8] and continuous (18 months) neuroleptic administration [4]. Furthermore, striatal neurotensin levels were decreased by long-term dopamine agonist treatment [4]. However, in contrast to enkephalins, neurotensin levels could also be modified after a single injection of dopamine antagonist drugs [6–8].

The existence of more than one type of dopamine receptor, and the frequent use of non-selective dopamine receptor agonist and antagonist drugs in such studies, may prevent identification of the receptor mediating a particular response. Biochemically, D-1 and D-2 dopamine receptor subtypes seem to mediate opposite effects as judged by alterations in cyclic AMP formation. Behaviourally a complex relationship exists with both D-1 and D-2 systems able to modulate motor behaviours initiated by selective occupation of one receptor subtype (see Ref. 9 for review). How these interactions relate to the ability of selective D-1 and D-2 receptor agonist and antagonist drugs to alter peptide levels is not known. Some previous studies have employed selective D-1 and D-2 dopamine receptor drugs to investigate alterations in basal

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ganglia peptide content. However, invariably these investigations have employed simple radioimmunoassay (RIA) measurements of neuropeptides in brain extracts [see for example 1–3, 6–8, 10]. Lack of completely specific antisera raises the possibility of cross-reactivity with structurally related peptides, peptide precursors/metabolites; or even with unknown substances within crude extracts making interpretation of the findings difficult.

The present study was designed to examine whether enkephalin and neurotensin levels are altered by acute administration of D-1 and D-2 dopamine receptor-selective agonist and antagonist drugs; and whether a functional relationship exists between D-1 and D-2 dopamine receptor-mediated changes in neuropeptide content. Peptide levels were assessed by a combined HPLC and RIA procedure to overcome problems of antisera specificity.

MATERIALS AND METHODS

Male Wistar rats (300–350 g; Bantin and Kingman) were housed in groups of six under standard conditions (12 hr light/dark cycle) at 21 ± 3° with food and water *ad libitum*. The following drugs were administered to rats: SKF 38393 (2.5–20 mg/kg), SCH 23390 (0.25–2.0 mg/kg), quinpirole (0.12–1.0 mg/kg) and sulpiride (25–100 mg/kg). Drug solutions were prepared by dissolving SKF 38393, quinpirole and SCH 23390 in distilled water. Dissolution of SCH 23390 was aided with a drop of 1% acetic acid. Sulpiride was dissolved in a minimum quantity of 2% sulphuric acid, made up to volume with distilled water, and adjusted to pH 7 with 1 M sodium hydroxide. All drugs were injected intraperitoneally in a fixed volume of 1 mL/kg of body weight. Control animals received corresponding amounts of vehicle. Animals received a single injection of drug or vehicle and were killed after a period of 1 hr for all drugs, except in the case of sulpiride, when 3 hr was employed to allow for its slow penetration into brain.

Peptide measurement. Rats were killed by cervical dislocation and decapitation. Brains were rapidly removed and dissected on an ice-cold plate. Dissected brain areas were immediately frozen on dry ice, weighed, and stored at −70° until biochemical determinations were made. The samples were boiled for 15 min in 100 vol. of a mixture 1 M acetic acid and 0.02 M hydrochloric acid, containing 0.1% 2-mercaptoethanol to prevent any oxidation of the peptides. Samples were homogenised and centrifuged (12,000 *g*, 10 min at 4°) and the supernatant was decanted. The tissue pellet was resuspended in 100 volumes of the acid mixture and centrifuged again. Aliquots (500 μL) of the combined supernatants (5 mg of wet weight tissue) were freeze-dried and stored at −40° until subjected to HPLC separation and subsequent radioimmunoassay.

The freeze-dried samples were reconstituted in HPLC buffer (250 μL), microfuged for 3 min and injected (200 μL) onto a Spherisorb 5 ODS 2 column (Phase Separations). Peptides were eluted with 0.5 M pyridine–0.5 M formic acid buffer, pH 4, containing 14% propan-1-ol at a flow rate of 1.0 mL/min. The chromatographic position of the native neuropeptides was determined by routinely separating a mixture of [^{3}H]peptides. Subsequently, the fractions (2 mL) obtained following separation of tissue samples, and which corresponded to the peaks for [^{3}H]peptides, were again freeze-dried and assayed by radioimmunoassay. The recovery of [^{3}H]neuropeptides from the chromatographic separation was always greater than 90%. The retention times for peptides were 7 min for [Met^5]-enkephalin, 14 min for [Leu^5]-enkephalin and 18 min for neurotensin. The freeze-dried residue of the HPLC mobile phase alone, when reconstituted with HPLC buffer, had no effect on the RIA for peptide analysis.

Specific RIA was used for peptide quantitation. In preliminary experiments competition curves with serial dilutions of the brain extracts after HPLC separation were examined to determine the appropriate sample dilution. The samples were reconstituted in an appropriate volume of buffer (50 mM sodium phosphate, pH 7.4, containing 0.2% gelatin and 10 mM ethyleneglycolbis(aminoethylether)tetraacetate (EDTA) for the enkephalins; for neurotensin RIA the assay buffer was 60 mM sodium phosphate, pH 7.2, containing 0.3% bovine serum albumin and 10 mM EDTA). An aliquot of samples or standards (100 μL) was incubated with 100 μL of antisera (Amersham International, Little Chalfont, Bucks, U.K.) and [^{125}I]peptide (15,000 cpm; Amersham International). All the samples were routinely assayed in duplicate. The tubes were incubated at 4° for 22–24 hours for [Met^5]-enkephalin, [Leu^5]-enkephalin and neurotensin to achieve appropriate sensitivity. Separation of bound and free peptide was achieved by adding 250–500 μL of activated charcoal-dextran in RIA buffer. After centrifugation (5000 *g*, 10 min) the supernatant was decanted, scintillation fluid added and samples counted in a Packard Tri-Carb liquid scintillation system, model A460 for 1 min. Cross reactivities of the antisera were as follows: the antisera did not display any cross-reactivity with dynorphin 1–13, [Met^5]-enkephalin-Arg-Phe, [Met^5]-enkephalin-Arg-Gly-Leu or CCK-8-S. Enkephalin antisera did not cross-react (< 0.1%) with antiserum against neurotensin, or *vice versa*. [Met^5]-enkephalin antiserum cross-reacted 6% with [Leu^5]-enkephalin, while [Leu^5]-enkephalin antiserum cross-reacted 5% with [Met^5]-enkephalin. The RIA sensitivities (15% displacement of bound tracer) were 1.8 fmol/tube for both enkephalins and 1.0 fmol/tube for neurotensin.

Drugs and chemicals. The sources of the compounds used in this study were as follows: SKF 38393 (*R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol hydrochloride; Smith Kline and French Laboratories, Philadelphia, PA, U.S.A.), SCH 23390 (2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-7,8-diol hydrochloride; Schering Corp., Bloomfield, NJ, U.S.A.), quinpirole (Lilly Research Laboratories, Indianapolis, IN, U.S.A.) and (±)-sulpiride (Laboratoires Delagrange, Paris, France).

Statistical analysis. Analysis of differences between means was by the unpaired Student's two-tailed *t*-test after single-factor analysis of variance (ANOVA) initially showed significant treatment effects. The

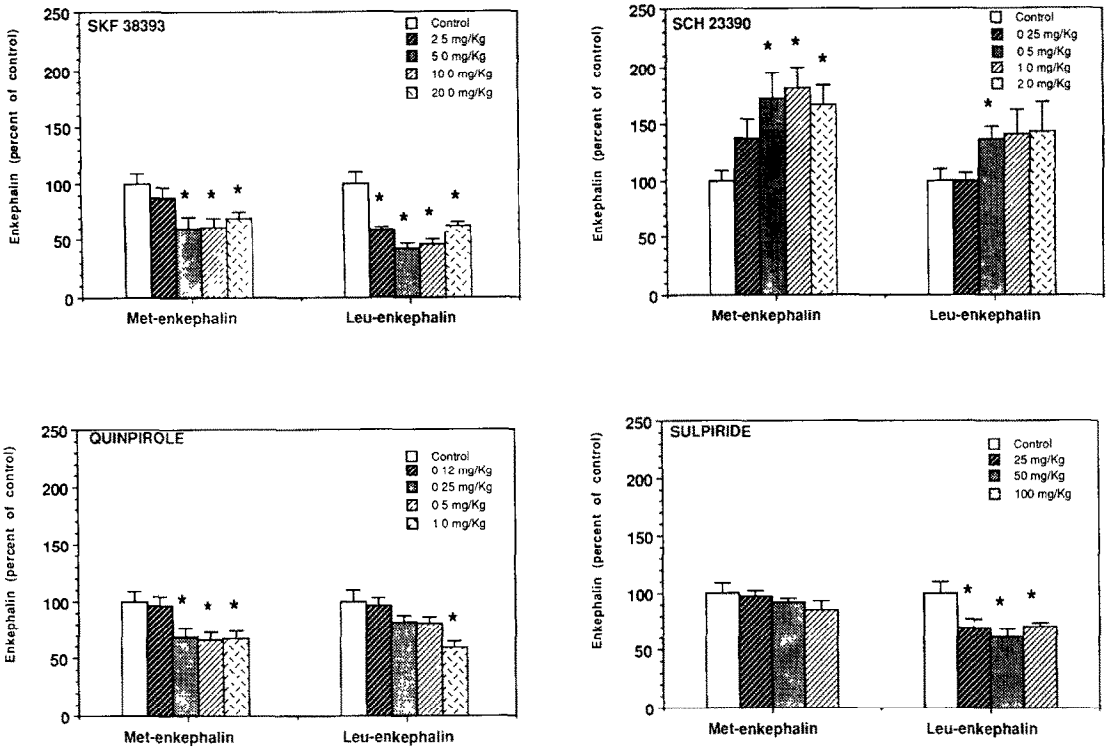

Fig. 1. Effects of acute administration of SKF 38393 (2.5–20.0 mg/kg), SCH 23390 (0.25–2.00 mg/kg), quinpirole (0.12–1.00 mg/kg) and sulpiride (25–100 mg/kg) on [Met^5]- and [Leu^5]-enkephalin content in nucleus accumbens. Rats were killed 1 hr after i.p. injection of the drugs, except in the case of sulpiride when rats were killed 3 hr after drug administration. The neuropeptides were measured using selective radioimmunoassays after HPLC separation of brain extracts. Each column represents mean ± SEM (of seven or eight animals), expressed as a percentage of control. The control values for [Met^5]- and [Leu^5]-enkephalin were 68.9 ± 6.4 and 16.5 ± 1.7 pmol/g tissue, respectively. Statistical significances were calculated by Student's two-tailed *t*-test, after single-factor analysis of variance initially showed significant treatment effects ($P < 0.001$ for SKF 38393, SCH 23390 and quinpirole effects on [Met^5]-enkephaline content and for SKF 38393 and sulpiride effects on [Leu^5]-enkephalin content; $P < 0.05$ for SCH 23390 and quinpirole effects on [Leu^5]-enkephalin content). * Significantly different $P < 0.05$, when compared with corresponding controls.

single-factor ANOVA was used to compare control mean neuropeptide concentration with the overall mean neuropeptide concentration at all the different doses of a particular drug.

RESULTS

[Met^5]- and [Leu^5]-enkephalin content in striatum, nucleus accumbens and substantia nigra

In striatum acute administration of SKF 38393 (2.5–20 mg/kg) or SCH 23390 (0.25–2.0 mg/kg) had no effect on [Met^5]- or [Leu^5]-enkephalin content (data not shown). Quinpirole (0.12–1.0 mg/kg) also had no effect. Sulpiride (25–100 mg/kg), as assessed by ANOVA, increased [Met^5]-enkephalin ($P < 0.05$) and [Leu^5]-enkephalin ($P < 0.01$) concentrations, although the effect of single doses was only statistically different from control at 25 mg/kg for [Leu^5]-enkephalin ($P < 0.05$). The control values for [Met^5]- and [Leu^5]-enkephalin were 503.4 ± 45.0 and 81.9 ± 3.9 pmol/g tissue respectively.

In nucleus accumbens the concentrations of both [Met^5]- and [Leu^5]-enkephalin were decreased by SKF 38393 (2.5–20 mg/kg) (Fig. 1). SCH 23390 (0.25–2.0 mg/kg) increased [Met^5]- and [Leu^5]-enkephalin concentrations in nucleus accumbens (Fig. 1). However, the effect of SCH 23390 on [Leu^5]-enkephalin concentration was only statistically different from control at 0.5 mg/kg. Quinpirole (0.12–1.0 mg/kg) decreased the levels of [Met^5]- and [Leu^5]-enkephalin in the nucleus accumbens; but the effect of quinpirole on [Leu^5]-enkephalin concentrations was only statistically different from controls at the highest dose used (1.0 mg/kg). [Met^5]-enkephalin concentrations in the nucleus accumbens were not altered by the administration of sulpiride, while the [Leu^5]-enkephalin was reduced by all doses of the drug (25–100 mg/kg).

In the substantia nigra [Met^5]-enkephalin concentrations were not modified by SKF 38393 (2.5–20 mg/kg) and SCH 23390 (0.25–2.0 mg/kg), or by quinpirole (0.12–1.0 mg/kg) or sulpiride (25–100 mg/kg) (Fig. 2). In contrast, [Leu^5]-enkephalin concentration in the substantia nigra was increased by SKF 38393 (10–20 mg/kg) and decreased by SCH 23390 (0.5–2.0 mg/kg), but quinpirole (0.12–1.0 mg/kg) and sulpiride (25–100 mg/kg) had no effect.

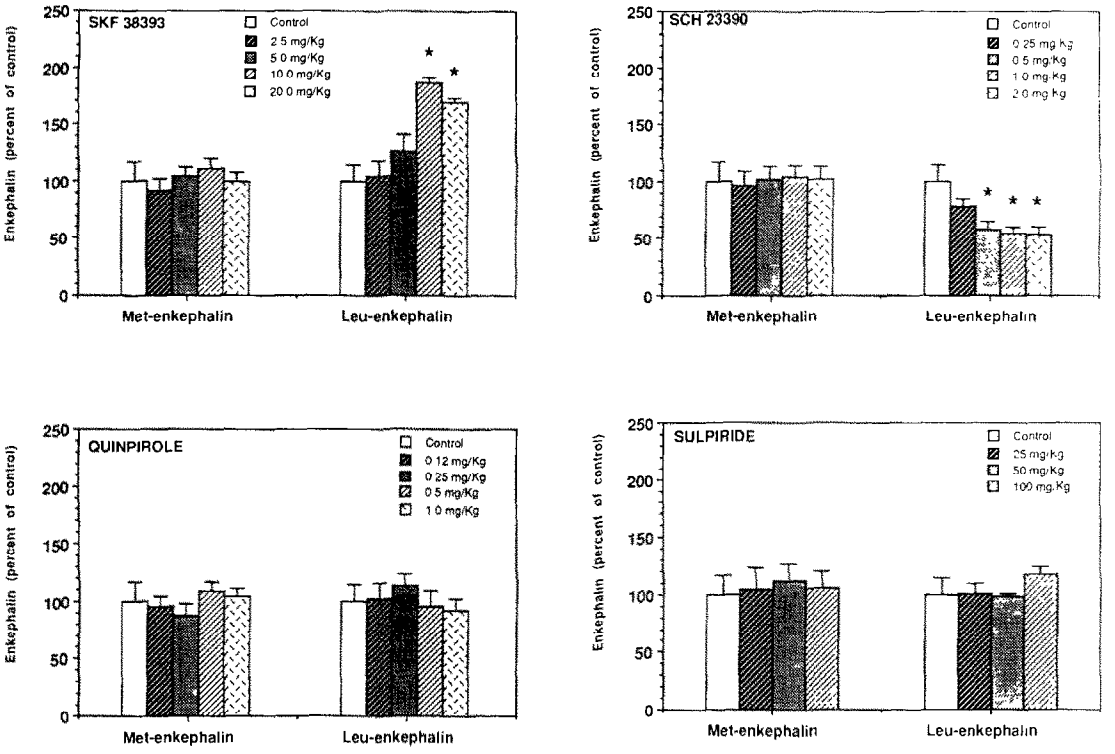

Fig. 2. Effects of acute administration of SKF 38393 (2.5–20.0 mg/kg), SCH 23390 (0.25–2.00 mg/kg), quinpirole (0.12–1.00 mg/kg) and sulpiride (25–100 mg/kg) on [Met^5]- and [Leu^5]-enkephalin content in substantia nigra. Rats were killed 1 hr after i.p. injection of the drugs, except in the case of sulpiride when rats were killed 3 hr after drug administration. The neuropeptides were measured using selective radioimmunoassays after HPLC separation of the brain extracts. Each column represents mean ± SEM (of seven or eight animals), expressed as a percentage of control. The control values for [Met^5]- and [Leu^5]-enkephalin were 61.4 ± 10.5 and 86.7 ± 12.1 pmol/g tissue, respectively. Statistical significances were calculated by a Student's two-tailed *t*-test, after single-factor analysis of variance initially showed significant treatment effects ($P < 0.001$ for SKF 38393 and $P < 0.05$ for SCH 23390 effects on [Leu^5]-enkephalin content). * Significantly different $P < 0.05$, when compared with corresponding controls.

Neurotensin content in striatum, nucleus accumbens and substantia nigra

In striatum neurotensin concentrations were increased after a single administration of SKF 38393 (10–20 mg/kg) and reduced after SCH 23390 (2.0 mg/kg) (Table 1). Quinpirole (1.0 mg/kg) also reduced neurotensin levels while they were increased following administration of sulpiride (50–100 mg/kg).

In nucleus accumbens neurotensin concentrations were not affected by SKF 38393 (2.5–20 mg/kg) but were increased by SCH 23390 (0.25–0.5 mg/kg). The increase was inversely correlated with the dose of SCH 23390 used and the neurotensin concentration was greater than 200% of the control concentration at 0.25 mg/kg (Table 1). Neither quinpirole (0.12–1.0 mg/kg) nor sulpiride (25–100 mg/kg) altered neurotensin levels in the nucleus accumbens.

In substantia nigra the neurotensin concentration was not modified by the acute administration of SKF 38393 (2.5–20 mg/kg) and SCH 23390 (0.25–2.0 mg/kg), or by quinpirole (0.12–1.0 mg/kg). Overall sulpiride (25–100 mg/kg) had no effect on neurotensin level in substantia although there was a trend for an increase at the 50 mg/kg dose level.

DISCUSSION

In the present study, striatal [Met^5]- and [Leu^5]-enkephalin levels were not modified by any drug treatment except sulpiride, which increased the concentration of both enkephalins, confirming our previous report [5]. This increase contrasts with another report detailing the absence of effect of sulpiride (and also SCH 23390) on striatal enkephalin content after acute administration [11]. These conflicting findings may be a consequence of the inclusion in the present work of a HPLC neuropeptide separation step prior to the RIA, compared with the direct RIA measurement of neuropeptides in brain extracts by Mocchetti *et al.* [11]. Recently, Sivam and colleagues [12] reported that [Met^5]-enkephalin levels in rat striatum were not altered by acute treatment with L-DOPA, quinpirole, SKF 38393 or SCH 23390 as assessed by direct RIA using doses of drugs similar to those employed in the present study. Since the acute administration of quinpirole, a D-2 dopamine receptor selective agonist, did not affect striatal enkephalin levels, it is possible that D-2 dopamine receptor occupation may not be implicated in the effect of sulpiride on striatal enkephalin content. This latter action may be specific to sulpiride

Table 1. Effect of acute administration of SKF 38393 (2.5–20.0 mg/kg), SCH 23390 (0.25–2.00 mg/kg), quinpirole (0.12–1.00 mg/kg) and sulpiride (25–100 mg/kg) on neurotensin content in basal ganglia

Treatment	Dose (mg/kg i.p.)	Striatum	Nucleus accumbens	Substantia nigra
Control		4.0 ± 0.4	14.5 ± 0.7	44.8 ± 6.0
SKF 38393	2.5	3.6 ± 0.5	13.8 ± 1.5	52.4 ± 3.5
	5.0	3.4 ± 0.4	16.8 ± 2.3	49.7 ± 4.0
	10	7.2 ± 0.6*	13.4 ± 1.2	55.9 ± 4.1
	20	7.8 ± 1.2*	15.1 ± 1.5	63.9 ± 4.6
SCH 23390	0.25	4.2 ± 0.3	34.3 ± 4.1*	48.1 ± 3.8
	0.5	4.3 ± 0.5	19.6 ± 1.4*	41.0 ± 2.8
	1.0	3.5 ± 0.3	17.9 ± 2.0	35.7 ± 3.6
	2.0	1.5 ± 2.0*	17.0 ± 1.9	34.7 ± 1.6
Quinpirole	0.12	4.1 ± 0.5	16.3 ± 2.2	51.0 ± 6.7
	0.25	4.3 ± 0.4	15.1 ± 1.7	42.9 ± 4.3
	0.5	4.0 ± 0.6	14.7 ± 1.1	42.5 ± 3.5
	1.0	2.5 ± 0.2*	13.8 ± 1.9	37.3 ± 5.1
Sulpiride	25	4.3 ± 0.4	14.6 ± 1.8	59.4 ± 9.9
	50	5.9 ± 0.4*	14.0 ± 1.5	74.4 ± 8.9
	100	5.7 ± 0.6*	14.0 ± 1.6	59.0 ± 7.0

Rats were killed 1 hr after i.p. injection of the drugs, except in the case of sulpiride when rats were killed 3 hr after drug administration. The neuropeptides were measured using selective radioimmunoassays after HPLC separation of the brain extracts. Results are mean ± SEM (of seven or eight animals). Statistical significances were calculated by a Student's two-tailed *t*-test, after single-factor analysis of variance initially showed a significant treatment effect ($P < 0.05$ for SKF 38393 and quinpirole, $P < 0.01$ for SCH 23390, and $P < 0.001$ for sulpiride effects on neurotensin content).

* Significantly different $P < 0.05$, when compared with corresponding control.

(and perhaps other substituted benzamides). Thus, there is apparently little acute dopaminergic regulatory influence over [Met^5]-enkephalin and [Leu^5]-enkephalin levels in the normal rat striatum.

In contrast to striatum a strong acute dopaminergic influence on [Met^5]-enkephalin and [Leu^5]-enkephalin in levels may exist in the normal rat nucleus accumbens. Interestingly, acute administration of a D-1 dopamine receptor selective agonist or of a D-1 antagonist had opposite effects. While SKF 38393 decreased [Met^5]- and [Leu^5]-enkephalin concentrations in the rat nucleus accumbens, SCH 23390 increased levels of both enkephalins. However, while the D-2 dopamine receptor selective agonist quinpirole also reduced concentrations of both pentapeptides in this area, sulpiride decreased only the [Leu^5]-enkephalin content in the nucleus accumbens. In the nucleus accumbens, acute stimulation of both dopamine receptor subtypes appeared to mediate similar effects on enkephalin levels. In the case of sulpiride, the effect on [Leu^5]-enkephalin was the same as that of SKF 38393, so it is possible that D-2 dopamine receptor blockade might unmask an action of endogenous dopamine on D-1 dopamine receptors. Alternatively, as discussed for striatum, this may reflect another action of sulpiride.

The concentrations of [Met^5]-enkephalin and [Leu^5]-enkephalin remained unchanged or were altered almost in parallel by acute administration of the D-1 and D-2 dopamine receptor selective drugs in both the striatum and nucleus accumbens. This is consistent with a report that they derive from a common precursor, proenkephalin A [13]. This was not the case in substantia nigra, where [Met^5]-enkephalin content was resistant to change after a single injection of dopamine receptor selective drugs. In contrast, [Leu^5]-enkephalin levels were increased by the D-1 dopamine receptor agonist SKF 38393 and decreased by the D-1 dopamine receptor antagonist SCH 23390. This action could be mediated by nigral D-1 dopamine receptors located presynaptically on striato-nigral projection neurones [14, 15]. [Leu^5]-enkephalin can derive both from pro-enkephalin A and prodynorphin precursors [16] but in substantia nigra it is likely to be formed from the latter [3, 17]. Indeed, the existence of a dynorphinergic striato-nigral pathway is well documented [17–19], making this hypothesis attractive. It is interesting to note the absence of an effect of D-2 dopamine agonist or antagonist drugs on enkephalin content in the substantia nigra. In fact, D-2 dopamine receptors in this area seem to be mainly localised to dopamine neurones in the zona compacta [15, 20], rather than on enkephalin-containing nigral efferents in the zona reticulata.

The alterations in enkephalin levels could be a consequence of alterations in [Met^5]- and [Leu^5]-enkephalin neuronal release and/or biosynthesis and degradation. They occurred a short time after acute drug administration (1–3 hr), so they are more likely to result from changes in release, rather than changes in peptide biosynthesis rate. In fact, dopamine enhances [Met^5]-enkephalin release from striatal slices, an effect that can be blocked by haloperidol [21]. Indeed, following pretreatment with selective

enkephalinase inhibitors such as tiorphan, others have also observed rapid increases in striatal [Met^5]- and/or [Leu^5]-enkephalin levels [22, 23]. Although Waksman and colleagues [24] were unable to detect changes in [Met^5]-enkephalin content in striatum following pretreatment with the mixed peptidase inhibitor kelatorphan. The turnover rate for peptides is relatively slow (typically 24 hr or longer) and it is unlikely that acute drug-induced peptide changes are due to changes in the peptide synthesis rate. However, hypothalamic proenkephalin mRNA content was changed 2 hr after a naloxone injection to induce opiate withdrawal [25]. Alternatively, changes in [Met^5]- and [Leu^5]-enkephalin levels may result from alterations in the activity of enkephalin degrative enzymes mediated by dopamine receptor selective drugs. D-1 and D-2 dopamine receptors located on enkephalin-containing neurones may mediate the effects observed. Indeed, the anatomical basis for such an interaction exists. There is evidence, at the ultrastructural level, of a direct dopamine input onto enkephalinergic neurones, at least in striatum [26]. Alternatively, the changes may be indirectly mediated through another neurone bearing both D-1 and D-2 receptor subtypes synapsing with an enkephalin-containing neurone. In striatum the interaction may occur via a cholinergic neurone since scopolamine administration partially prevents the increase in striatal enkephalin content elicited by chronic haloperidol administration [27].

For neurotensin, the most prominent effects following acute administration of dopamine receptor selective drugs occurred in the striatum. D-1 and D-2 dopamine receptor selective agonists and antagonists had opposite effects. These results are consistent with the report from Frey *et al.* [7], who studied striatal neurotensin content 6–48 hr after a single injection of SCH 23390 and sulpiride. Merchant *et al.* [10], similarly found that D-1 and D-2 dopamine receptors mediated an antagonistic regulation of neurotensin levels in striatum following multiple drug administration. Merchant *et al.* also observed similar actions in the nucleus accumbens, but these were not seen in the present study where tissues were examined much earlier following drug administration. The mechanism responsible for dopamine receptor-mediated alterations in neurotensin content is at present unknown. Dopaminergic-induced modification of neurotensin neuronal release could be responsible for changes in content. A decrease in striatal neurotensin release has been observed in the presence of haloperidol [8], suggesting that increased neurotensin content is a consequence of decreased neuropeptide utilisation. In conclusion, a strong dopaminergic regulatory influence exists over neurotensin levels in the normal rat striatum. D-1 and D-2 dopamine receptors appeared to have opposing roles, consistent with earlier biochemical work [28], in mediating the regulation of striatal neurotensin levels.

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