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Opiate-receptor mediated changes in monoamine synthesis in rat brain

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The effects of morphine, β-endorphin, naloxone and naltrexone on the rate of tyrosine and tryptophan hydroxylation were investigated *in vivo* by measuring the accumulation of dopa and 5-hydroxytryptophan (5-HTP) in different brain regions of rats after inhibition of the aromatic L-amino acid decarboxylase. The cerebral concentrations of tyrosine and tryptophan were also measured. Morphine (3–30 mg kg^{-1}) increased the accumulation of dopa dose-dependently (25–50%) in the dopamine-rich areas (limbic forebrain and corpus striatum). In the noradrenaline-predominant parts of the brain (containing hemispheres, diencephalon and lower brain stem) only the highest dose of morphine (30 mg kg^{-1}) significantly increased dopa formation (47%). Similarly to morphine, intracerebroventricularly injected β-endorphin (5–10 μg per rat) increased the formation of dopa. This increase was doubled in limbic forebrain, corpus striatum and cerebral hemispheres. Doses of 10 to 20 μg of β-endorphin were needed to increase dopa accumulation in the diencephalon and the lower brain stem. Naloxone antagonized the β-endorphin-induced increases in dopa. But naloxone and naltrexone (10–100 mg kg^{-1}) decreased the dopa formation in the dopamine-rich areas (about 20–25%) but not in the noradrenaline-predominant areas. Morphine (30 mg kg^{-1}) and β-endorphin (5 μg per rat) increased the accumulation of 5-HTP whereas naloxone and naltrexone (10 mg kg^{-1}) tended to decrease its formation. Morphine and β-endorphine increased the concentrations of tyrosine and tryptophan, and naloxone decreased the cerebral tryptophan concentration. These results show that the effects of a narcotic agonist (morphine) and of pure narcotic antagonists (naloxone and naltrexone) on the synthesis of dopamine and 5-HT are opposite to each other. Furthermore, the effects of β-endorphine on brain monoamine synthesis are remarkably similar to those of morphine. Thus, it is probable that opiate receptors and their endogenous ligands are involved in the regulation of dopamine and 5-HT synthesis.

Recently peptides with morphine-like properties have been isolated from the brain and characterized. These peptides are thought to be the endogenous ligands for opiate receptors (see Kosterlitz, 1976). It is well known that morphine and other narcotic analgesics increase the turnover of dopamine in the brain and that naloxone antagonizes these effects (Kuschinsky, 1976). Therefore it can be anticipated that the morphine-like neuropeptides would have similar effects and it is likely that opiate receptors and their endogenous ligands could be involved in the regulation of dopamine neurons.

However, there are no reports about the effects of pure narcotic antagonists on brain monoamine synthesis in intact animals. This fact prompted us to study the possible functional connection between opiate receptors and monoaminergic systems. The effects of morphine and the pure narcotic antagonists, naloxone and naltrexone, on the rate of tyrosine and tryptophan hydroxylation were investigated *in vivo* by measuring the accumulation of dopa and 5-hydroxytryptophan in different brain regions of rats treated with an inhibitor of L-aromatic amino acid decarboxylase (Carlsson, Davis & others, 1972). High doses of narcotic antagonists were used to block the opiate receptors as completely as possible.

In addition, we studied the effects of intracerebroventricularly injected β-endorphin on brain monoamine synthesis. β-Endorphin is *in vivo* the most potent of the endogenous morphine-like peptides (Bloom, Segal & others, 1976; Jacquet & Marks, 1976; Wei, Tseng & others, 1977). Owing to the small amount of β-endorphin available, only a limited number of experiments could be done. We chose to assess dose-response and time-response relations for the effects of β-endorphin and also to study the combined effect of naloxone and β-endorphin. The ratio between the doses of β-endorphin inducing catalepsy and those affecting brain-dopamine metabolism was similar to that found for morphine and this fact helped us to design our experiments adequately.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, 200–270 g (Anticimex, Stockholm), receiving a standard diet with water

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freely available, were housed at 24° under a 12 h light–dark cycle.

Drugs

Morphine HCl (Pharmacopoea Nordica), naloxone HCl and naltrexone HCl (Endo Lab., Garden City, N.Y.) and 3-hydroxybenzylhydrazine HCl (NSD 1015, synthesized in this institute by Dr P. Lindberg) were dissolved in 0·9% w/v NaCl solution (saline) and injected intraperitoneally (i.p.). Doses of morphine, naloxone and naltrexone refer to the free base. Synthetic human β-endorphin (β-lipotropin$_{61-91}$ fragment; gift from Prof. C. H. Li) was dissolved in saline and injected intracerebroventricularly. The time and dose schedules for injections are given in the Results section. Control rats always received the same number of injections of saline at the same time intervals as the experimental animals.

Implantation of intracerbroventricular (i.c.v.) cannulae

The animals were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.p.) and their skulls were exposed by means of a midline incision. A hole was drilled on each side of the skull 2 mm posterior to the bregma and 2 mm lateral from the sagittal suture. Polyethylene cannulae (made from Portex polyethylene tubing (o.d. 0·75, i.d. 0·30 mm) were implanted through these holes so that they reached the lateral ventricles 4 mm below the surface of the skull. The cannulae were fixed by means of acrylic dental cement supported by a metallic screw on each side of the skull. The experiments were performed two days after the operation when the gross behaviour of the animals was normal.

At the time for the experiment, β-endorphin was injected in a dose of 1·25 to 10 μg into the right lateral ventricle, followed within 1 min by the same dose into the left lateral ventricle (10 μl β-endorphin solution followed by 5 μl saline into each ventricle). Control rats were injected with 15 μl of saline into each ventricle. The proper position of the cannulae was checked at autopsy.

Synthesis of brain monoamines

The brain monoamine synthesis was studied by measuring the accumulation of 3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP) for 30 min after inhibition of the aromatic L-amino acid decarboxylase by a supramaximal dose of NSD 1015 (3-hydroxybenzylhydrazine) (100 mg kg^{-1}) (Carlsson & others, 1972).

Dissection of the brain

The rats were decapitated between 11·00 and 13·00 h. The brains were quickly removed and dissected on an ice-cold plate into the following parts: (1) limbic forebrain containing e.g. the olfactory tubercle, nucleus accumbens (medial part), nucleus amygdaloideus centralis and part of the paleocortex, (2) corpus striatum, (3) the remainder of the hemispheres including hippocampus (referred to as hemispheres), (4) diencephalon and (5) lower brain stem. In some experiments, parts (3), (4) and (5) were pooled (referred to as rest). Cerebellum and pineal were always discarded. Dopamine is the predominating catecholamine in parts (1) and (2) and noradrenaline in parts (3), (4) and (5). For details of dissection see Carlsson & Lindqvist (1973).

Estimation of dopa, 5-HTP, tyrosine and tryptophan

Immediately after dissection the brain parts were frozen on dry ice. The parts of 2 brains were pooled, weighed and stored at −80°. After thawing the pooled brain parts were homogenized in 10 ml 0·4 M perchloric acid containing 5 mg sodium metabisulphite and 20 mg EDTA. The homogenates were centrifuged at about 10 000 *g* for 10 min at 0° and the supernatant purified on a strong cation exchange column (Dowex 50) (Kehr, Carlsson & Lindqvist, 1972; Atack & Magnusson, 1978). The following spectrophotofluorimetric analyses were made: dopa (Kehr & others, 1972), 5-HTP (Atack & Lindqvist, 1973), tyrosine (Waalkes & Udenfriend, 1957) and tryptophan (Bédard, Carlsson & Lindqvist, 1972).

Statistics

Student's *t*-test or one way analysis of variance followed by Student's *t*-test were used. The level of significance was chosen as $P = 0{\cdot}05$.

RESULTS

Effect of morphine on brain monoamine synthesis

As Table 1 shows, morphine, 1 h after administration at 3, 10 an 30 mg kg^{-1}, enhanced the formation of dopa, but not that of 5-HTP, in the brain of rats treated with the aromatic L-amino acid decarboxylase inhibitor, NSD 1015. This effect was clearly dose-dependent in the dopamine-rich areas, i.e. the limbic forebrain and the corpus striatum. But in the noradrenaline-predominant parts of the brain (rest) only the highest dose of morphine (30 mg kg^{-1}) significantly increased dopa formation. Morphine (3–30 mg kg^{-1}) in 1 h induced no changes in tyrosine concentrations in any of the brain regions studied, but tryptophan concentrations increased by 30–40% ($P < 0{\cdot}05$) in the rest of the brain (data not shown).

Two hours after the administration of morphine

(30 mg kg^{-1}) (Table 2) its effect on the dopa formation in the limbic forebrain and the rest of the brain was slightly less than after 1 h. However, in the corpus striatum the dopa formation 2 h after morphine was clearly higher than after 1 h and almost twice as fast as in the brain of the control rats. At 2 h 5-HTP formation and tyrosine concentrations tended to increase in all brain regions but the difference was significant only in the rest of the brain (5-HTP by 34%; tyrosine by 71%). Tryptophan concentrations were slightly but significantly increased in all brain regions.

Table 1. *Dopa and 5-hydroxytryptophan (5-HTP) formation in rat brain regions 1 h after administration of various doses of morphine.* Morphine was injected intraperitoneally 30 min before NSD 1015 (100 mg kg^{-1}, i.p.) and the animals were killed after another 30 min. Controls received the same volume of saline before NSD 1015 as above. Shown are the means (ng g^{-1}) ± s.e.m. of 3 determinations. Statistical significances were calculated according to one way analysis of variance followed by *t*-test. Differs from control: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.005$; $^{d}P < 0.001$.

Morphine (mg kg^{-1})	Dopa			5-HTP		
	Limbic	Striatum	Rest	Limbic	Striatum	Rest
Controls	389 ±23	541 ±36	100 ±6	97 ±6	53 ±4	78 ±5
3	481 ±21^{a}	681 ±25^{b}	107 ±5	101 ±2	51 ±6	81 ±5
10	541 ±40^{c}	757 ±32^{d}	122 ±6	100 ±7	66 ±4	85 ±6
30	591 ±16^{d}	619 ±20	147 ±15^{b}	109 ±6	62 ±2	89 ±6

Effect of β-endorphin on brain monoamine synthesis The intracerebroventricular (i.c.v.) injections of β-endorphin (2·5–20 μg per rat) increased the formation of dopa in all brain regions studied (Fig. 1). The lower doses (2·5 and 5 μg per rat) increased its accumulation in the limbic forebrain (40%; $P < 0.01$ and 67%; $P < 0.001$, respectively), corpus striatum (14%; N.S. and 45%; $P < 0.005$, respectively) and hemispheres (39%; $P < 0.05$ and 77%;

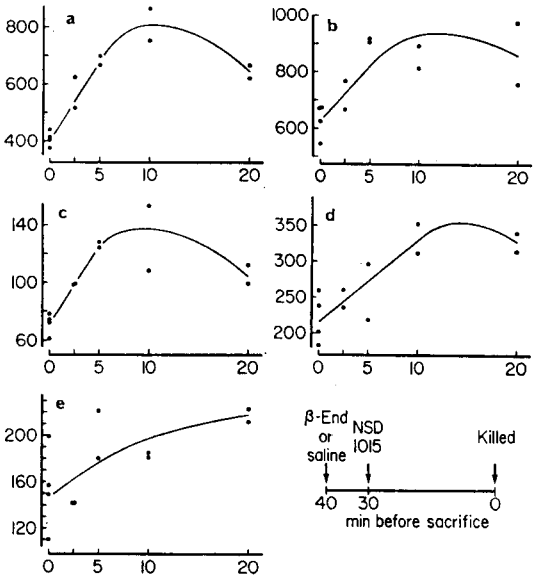

FIG. 1. Dose-response curves for the effect of β-endorphin (μg per rat) (abscissa) on dopa formation (ng g^{-1}) (ordinate) in rat brain regions. β-Endorphin was injected intracerebroventricularly 10 min before NSD 1015 (3-hydroxybenzylhydrazine, 100 mg kg^{-1}, i.p.) and the rats were killed after another 30 min. Control rats received the same volume of saline (i.c.v.) 10 min before the NSD 1015. Each point represents 2 pooled brain parts. a: Limbic; b: striatum; c: hemispheres; d: diencephalon; e: brainstem.

Table 2. *The synthesis of monoamines and the concentrations of tyrosine and tryptophan in rat brain regions. 2 h after administration of morphine.* Morphine (30 mg kg^{-1}, i.p.) was injected 90 min before NSD 1015 (100 mg kg^{-1}, i.p.) and the animals were killed after another 30 min. Controls received the same volume of saline before NSD 1015 as above. Shown are the means ± s.e.m. of 3 determinations. Statistical significances were calculated according to Student's *t*-test. Differs from control: $^{a}P < 0.05$; $^{b}P < 0.025$; $^{c}P < 0.01$; $^{d}P < 0.005$.

Brain region	Dopa ng g^{-1}		5-HTP ng g^{-1}		Tyrosine μg g^{-1}		Tryptophan μg g^{-1}	
	Control	Morphine	Control	Morphine	Control	Morphine	Control	Morphine
Limbic	451 ± 23	620 ± 44^{a}	113 ± 3	122 ± 3	22 ± 1	28 ± 3	5·1 ± 0·2	5·9 ± 0·1^{c}
Striatum	541 ± 21	982 ± 73^{d}	52 ± 3	81 ± 12	21 ± 1	30 ± 3	4·9 ± 0·1	6·0 ± 0·3^{c}
Rest	101 ± 7	131 ± 4^{a}	88 ± 7	118 ± 4^{c}	21 ± 2	36 ± 3^{b}	4·0 ± 0·2	5·0 ± 0·1^{d}

P <0·005, respectively) but higher doses (10–20 μg per rat) were needed to cause a significant increase in the diencephalon (50%; P <0·01 and 48%; P <0·01, respectively) and in the lower brain stem (20%; N.S. and 41%; P <0·05, respectively). As the figures show, the dose-response curves for this effect seem to be approximately linear up to 5 μg per rat in the corpus striatum (maximum increase 45%; P <0·005), in the limbic forebrain (maximum increase 100%; P <0·001), in the hemispheres (maximum increase 85%; P <0·005) and in the diencephalon (maximum increase 50%; P <0·01). After the maximum effect was reached in these four brain regions a further increase in the dose of β-endorphin up to 20 μg per rat induced a more or less clearcut decline of the dose-response curves. In the lower brain stem the dose-response relationship was uncertain because of the scatter of the control values. Fig. 2 shows the time-response relation of the effect of a single dose (5 μg per rat) of β-endorphin on dopa formation in rat brain regions. In the dopamine-rich areas, i.e. the limbic forebrain and corpus striatum, the maximum increase in dopa formation occurred slightly later than in the noradrenaline predominant parts, i.e. the cerebral hemispheres. The maximum increase in the limbic forebrain was reached at 1 h after intracerebroventricular injection of β-endorphin (45%; P <0·05) and was still apparent after 1·5 h (~45%; P <0·05). With the corpus striatum this maximum effect was reached at 1 h (100%; P <0·005) and after 1·5 and 2 h it was still increased by 60% (P <0·025) and in the hemispheres it was significantly increased at 40 min (57%; P <0·025) and at 1 h (46%; P <0·05) after β-endorphin administration.

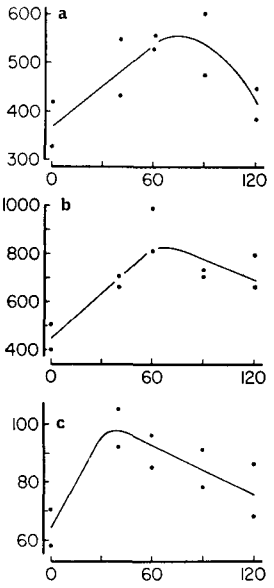

FIG. 2. Time-response relation of the effect of a single dose (5 μg per rat) of β-endorphin on dopa formation (ng g^{-1}) (ordinate) in rat brain regions. β-Endorphin was injected intracerebroventricularly at zero time and NSD 1015 (100 mg kg^{-1}, i.p.) 30 min before the rats were killed. Control rats received the same volume of saline (i.c.v.) 10 min before the NSD 1015. Each point represents 2 pooled brain parts. a: Limbic; b: striatum; c: hemispheres. Abscissa: Time (min after β-endorphin).

As could be anticipated from the dose-response curves, 5 μg of β-endorphin per rat did not induce changes in formation of dopa in the diencephalon and the lower brain stem at any time (data not shown).

The increase in dopa formation induced by β-endorphin (5 μg per rat) was antagonized by naloxone (10 mg kg^{-1}) in all brain regions (Fig. 3), even though the increase induced was not statistically significant in all brain regions.

5-HTP formation was not significantly increased in any of the brain regions studied 40 min after the intracerebroventricular injections of β-endorphin (2·5–20 μg per rat) (data not shown). However, at later times, β-endorphin (5 μg per rat) induced significant increases in 5-HTP formation in all brain regions except hemispheres. The maximum increases (50–80%) were reached at 1·5–2 h after β-endorphin (Table 3).

Table 4 shows the dose-response and time response relations of the effect of β-endorphin on tyrosine concentrations in rat brain regions, doses of 2·5–20 μg i.c.v. per rat did not change the tyrosine concentrations 40 min after administration. However by 2 h, there were significant increases (30–60%) in all brain regions studied. These results show that β-endorphin-induced changes in brain tyrosine concentrations do not appear before 1 h after its intracerebroventricular administration.

β-Endorphin (2·5–20 μg i.c.v., per rat) did not induce changes in tryptophan concentrations by 40 min (data not shown). However, by 2 h there were significant increases (35–40%) in all brain regions (Table 5). β-Endorphin-induced changes in tryptophan concentrations occurred clearly later than those of tyrosine determined in the same brain samples.

Effect of naloxone and naltrexone on brain monoamine synthesis

As Table 6 shows, naloxone, 1 h after its administration in the doses of 10, 30 and 100 mg kg^{-1},

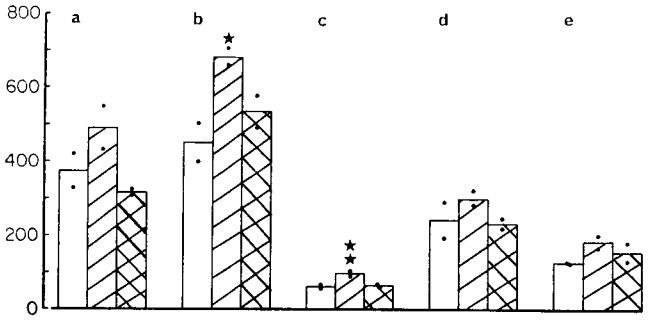

FIG. 3. Reversal by naloxone of the β-endorphin-induced increases in dopa formation (ng g^{-1}) (ordinate) in rat brain regions. β-Endorphin (5 μg per rat) was injected intracerebroventricularly 10 min before NSD 1015 (100 mg kg^{-1}, i.p.) and the rats were killed after another 30 min. Control rats received the same volume of saline (i.c.v.) 10 min before the NSD 1015. Naloxone (10 mg kg^{-1}) was given subcutaneously 1 min before β-endorphin. Each point represents 2 pooled brain parts. * P <0·05; ** P <0·025 (One way analysis of variance followed by t-test). a: Limbic; b: striatum; c: hemispheres; d: diencephalon; e: brainstem. Open columns: controls; hatched columns: β-endorphin; cross-hatched columns: naloxone plus β-endorphin.

decreased the formation of dopa in the dopamine-rich areas but not in the noradrenaline-predominant areas. However, no apparent dose-response relation was seen with the doses used because 10 mg kg^{-1} already caused the maximum decrease (about 20–25%). The dose of 10 mg kg^{-1} decreased slightly the formation of 5-HTP in the corpus striatum. Naloxone (30 mg kg^{-1}) at 1 h significantly decreased the tryptophan concentrations in the limbic forebrain (by 13%; P <0·05) and in the rest of the brain (by 20%; P <0·01) (data not shown).

Table 7 shows that even in a lower dose (1 mg kg^{-1}) and after a longer time (90 min) naloxone still decreased dopa and 5-HTP formation in the limbic forebrain and tryptophan concentrations in the corpus striatum.

Naltrexone, another relatively pure opiate antagonist with the same pharmacological proper-

Table 3. *Time-response relation of the effect of β-endorphin on 5-HTP formation in rat brain regions.* β-Endorphin (5 μg per rat, i.c.v.) was injected at different times and NSD 1015 (100 mg kg^{-1}, i.p.) 30 min before the rats were killed. Time refers to the interval between the administration of β-endorphin and death. Control rats received the same volume of saline (i.c.v.) 10 min before NSD 1015. Shown are the means of 4 (control) or 2 (experimental) determinations (two pooled brain parts). Statistical significances were calculated according to one way analysis of variance followed by t-test. Differs from control: [a] P <0·05; [b] P <0·025; [c] P <0·01; [d] P <0·001.

	5-HTP (ng g^{-1} ± s.e.)				
Brain region	Control	40 min	60 min	90 min	120 min
Limbic regions	75 ±3	85 ±5	105 ±6[b]	134 ±3[d]	108 ±10[c]
Corpus striatum	49 ±5	61 ±0	61 ±2	71 ±11	75 ±2[b]
Hemispheres	60 ±13	60 ±5	71 ±11	84 ±4	65 ±4
Diencephalon	116 ±13	133 ±3	138 ±3	173 ±10[b]	160 ±19[a]
Lower brain stem	139 ±7	164 ±10	179 ±11	197 ±24[a]	210 ±10[b]

Table 4. *The effect of β-endorphin on the concentration of tyrosine in rat brain regions* (Other details (except β-endorphin dosage) are as in Table 3.

		Tyrosine (μg g^{-1} ± s.e.)				
β-Endorphin (μg per rat)	Time (min)	Limbic regions	Striatum	Hemispheres	Diencephalon	Lower brain stem
Control	—	18 ±0·4	19 ±2	21 ±2	17 ±1	16 ±1
2·5	40	18 ±0·7	19 ±2	21 ±0·1	18 ±0·4	14 ±1
5	40	18 ±0·5	18 ±2	20 ±2	20 ±4	18 ±2
10	40	17 ±2	18 ±1	21 ±3	20 ±3	14 ±1
20	40	18 ±1	18 ±1	22 ±6	17 ±1	15 ±1
Control	—	17 ±2	17 ±2	17 ±1	19 ±0·4	16 ±1
5	60	23 ±1[a]	19 ±0·2	24 ±2	20 ±0·6	20 ±0·3[a]
5	90	25 ±1[b]	20 ±2	27 ±3[b]	24 ±1[b]	23 ±2[b]
5	120	18 ±0·4	23 ±0·5[a]	22 ±1	22 ±1[a]	19 ±1

Table 5. *Time-response relation of the effect of β-endorphin on the concentration of tryptophan in rat brain regions.* Other details as for Table 3.

Brain region	Tryptophan (μg g^{-1} ± s.e.) Control	40 min	60 min	90 min	120 min
Limbic regions	4·5 ±0·4	4·7 ±0·3	4·6 ±0·1	5·9 ±0·8	6·3 ±0·2[b]
Corpus striatum	4·5 ±0·4	4·9 ±0·1	4·6 ±0·1	5·5 ±0·5	6·3 ±0·2[c]
Hemispheres	4·6 ± 0·2	5·1 ±0·5	5·3 ±0·6	5·8 ±0·4	6·5 ±0·1[b]
Diencephalon	5·0 ±0·1	5·5 ±0·0	5·6 ±0·1	6·1 ±0·5[a]	6·8 ±0·3[d]
Lower brain stem	4·3 ±0·3	5·0 ±0·4	4·9 ±0·4	5·5 ±0·7	6·1 ±0·2[a]

ties as naloxone but with a longer duration of action, decreased the formation of dopa in the limbic forebrain and the corpus striatum to about the same extent as naloxone (Table 8). Like naloxone, naltrexone did not decrease dopa formation in the rest of the brain where the predominant catecholamine is noradrenaline. In this brain region, naltrexone (10 mg kg^{-1}) significantly decreased 5-HTP formation. No effect was seen on tryptophan concentration in any of the brain regions (data not shown).

Behaviour of the animals

Morphine (3–30 mg kg^{-1}) induced a loss of both the corneal reflex and the tail-pinch reflex. The lower dose of morphine (3 mg kg^{-1}) increased the activity of the rats (sniffing, chewing and locomotion) and the higher dose (30 mg kg^{-1}) induced clear catatonia.

β-Endorphin in a dose of 2·5 μg per rat did not induce any clear changes in behaviour, however, higher doses (5–20 μg per rat) caused a loss of corneal reflex, exophthalmus and induced salivation and catatonia in less than 10 min. When the time course of the effect of a single dose (5 μg per rat) of β-endorphin was investigated, these effects lasted less than 1 h. In naloxone-pretreated rats none of these effects appeared.

Table 6. *Dopa and 5-hydroxytryptophan (5-HTP) formation in rat brain regions 1 h after administration of various doses of naloxone.* Naloxone was injected intraperitoneally 30 min before NSD 1015 (100 mg kg^{-1}, i.p.) and the animals were killed after another 30 min. Controls received the same volume of saline before NSD 1015 as above. Shown are the means (ng g^{-1}) ± s.e.m. of 3–5 determinations. Statistical significances were calculated according to one way analysis of variance followed by *t*-test. Differs from control: [a]P <0·05; [b]P <0·025.

Naloxone (mg kg^{-1})	Dopa Limbic	Striatum	Rest	5-HTP Limbic	Striatum	Rest
Controls	452 ±10	598 ±49	95 ±6	105 ±2	53 ±2	75 ±1
10	384 ±20[b]	453 ±13[b]	97 ±1	91 ±7	44 ±2[a]	76 ±7
30	379 ±25[b]	491 ±24[a]	96 ±8	96 ±3	48 ±3	72 ±4
100	384 ±23[b]	527 ±30	86 ±4	115 ±4	52 ±2	75 ±4

Rats treated with 10 and 30 mg kg^{-1} of opiate antagonists naloxone and naltrexone appeared slightly sedated and after the highest dose (100 mg kg^{-1}) some of the animals were slightly cataleptic.

DISCUSSION

The present results show that the effects of a narcotic agonist, morphine, and of pure narcotic antagonists, naloxone and naltrexone, on the formation of dopa in dopamine-rich areas of rat brain were opposite to each other. Thus, it seems plausible that the

Table 7. *Effect of naloxone (Nal.) on the synthesis of monoamines and on tyrosine and tryptophan concentrations in rat brain regions.* Naloxone (1 mg kg^{-1}, i.p.) was injected 60 min before NSD 1015 (100 mg kg^{-1}, i.p.) and the animals were killed after another 30 min. Controls received the same volume of saline before NSD 1015 as above. Shown are the means ± s.e.m. of 6 determinations. Statistical significances were calculated according to Student's *t*-test. Differs from control: [a]P <0·05; [b]P <0·025.

Brain region	Dopa ng g^{-1} Cont.	Nal.	5-HTP ng g^{-1} Cont.	Nal.	Tyrosine μg g^{-1} Cont.	Nal.	Tryptophan μg g^{-1} Cont.	Nal.
Limbic	307 ± 8	251 ± 19[b]	160 ± 23	89 ± 9[b]	22 ± 2	21 ± 1	4·9 ± 0·2	4·9 ± 0·4
Striatum	414 ± 32	385 ± 20	72 ± 6	58 ± 8	21 ± 2	18 ± 1	4·9 ± 0·3	4·0 ± 0·1[a]
Hemispheres	38 ± 3	38 ± 5	57 ± 3	52± 2	19 ± 2	18 ± 1	3·8 ± 0·1	3·7 ± 0·2

opiate receptors are involved in the regulation of cerebral dopamine synthesis. Similarly to morphine, β-endorphin enhanced the formation of dopa and naloxone antagonized this increase. Therefore it is probable that opiate receptors mediate this effect.

The dose of 2·5 μg of β-endorphin per rat did not induce any clear changes in behaviour but it did increase the formation of dopa. Morphine at a dose (3 mg kg^{-1}) which did not induce catalepsy, enhanced the dopa formation. The largest β-endorphin-induced increases in dopa formation occurred in the limbic forebrain, the corpus striatum and the cerebral hemispheres, in which the doses of 5 or 10 μg per rat of β-endorphin about doubled the dopa formation. Increasing the dose of β-endorphin to 20 μg per rat did not cause a further increase, on the contrary this dose caused a smaller increase than 10 μg, in these three brain regions which are situated near the lateral ventricles. However, in the lower brain stem, which among the five brain regions examined is the most distant one from the lateral ventricles, the 20 μg per rat dose at first significantly increased the dopa formation. It remains to be seen to what extent the distance from the site of administration or from the csf influences the results. Thus our experiment shows that β-endorphin, similarly, to morphine, increases the formation of dopa in all parts of the brain and the enhancement seems to last longest in the corpus striatum both in the morphine-treated and in the β-endorphin-treated rats.

Table 8. *Dopa and 5-hydroxytryptophan (5-HTP) formation in rat brain regions 1 h after administration of various doses of naltrexone.* Naltrexone was injected intraperitoneally 30 min before NSD 1015 (100 mg kg^{-1}, i.p.) and the animals were killed after another 30 min. Controls received the same volume of saline before NSD 1015 as above. Shown are the means (ng g^{-1}) ± s.e.m. of 3 determinations. Statistical significances were calculated according to one way analysis of variance followed by *t*-test. Differs from control: $^{a}P < 0{\cdot}05$; $^{b}P < 0{\cdot}025$; $^{c}P < 0{\cdot}01$.

Naltrexone (mg kg^{-1})	Dopa			5-HTP		
	Limbic	Striatum	Rest	Limbic	Striatum	Rest
Controls	393 ±15	516 ±42	83 ±6	106 ±6	64 ±6	93 ±3
10	324 ±24^{b}	386 15^{b}	98 ±3	115 ±2	57 ±1	78 ±3^{a}
30	308 ±15^{c}	394 ±20^{b}	84 ±3	113 ±3	67 ±2	91 ±3
100	381 ±4	437 ±29	95 ±5	101 ±8	62 ±4	84 ±7

The present results show that β-endorphin has morphine-like effects on the formation of catecholamines. It can be speculated that β-endorphin and/or other endogenous opiate-like peptides could be involved in the regulation of cerebral catecholamine synthesis. Such a suggestion is supported by the observations that the rate of synthesis of catecholamines from labelled tyrosine in the brain of morphine-tolerant mice (Rosenman & Smith, 1972), and the conentration of homovanillic acid in the striatum of methadone-tolerant rats (Ahtee, 1974), are decreased. It is possible that chronic treatment with the narcotic analgesics produces tolerance to the stimulating action of endogenous opiate-like peptides on dopa formation; chronic morphine treatment produces tolerance towards the antinociceptive, catatonic and hypothermic effects of exogenous β-endorphin (Tseng, Loh & Li, 1977).

The highest extent to which dopa formation was activated by morphine and by β-endorphin was less than twice the controls in the corpus striatum. This finding agrees well with the findings that striatal homovanillic (HVA) and dihydroxyphenylacetic (DOPAC) acid concentrations are about doubled by narcotic analgesics (Gessa & Tagliamonte, 1975; Kääriäinen & Ahtee, 1976). On the other hand, the neuroleptic drugs cause three to four fold increases in dopa formation (Carlsson, Kehr & Lindqvist, 1977) and in the concentrations of HVA and DOPAC (Gessa & Tagliamonte, 1975; Kääriäinen & Ahtee, 1976). In our study, naloxone and naltrexone decreased dopa formation by about 20–25% in the limbic forbrain and in the corpus striatum. Thus their effects were weaker than, for example, that of apomorphine (Carlsson & others, 1977). These findings indicate that the efficacy of drugs acting on the opiate receptors in controlling the synthesis of dopamine is lower than that of drugs acting directly on dopamine receptors. However, several other receptors mediate changes in the synthesis and release of cerebral dopamine, e.g. the muscarinic and nicotinic cholinoceptors, and drugs acting on these receptors alter the metabolism of dopamine less than drugs acting on dopamine receptors do (Javoy, Agid & Glowinski, 1975; Ahtee & Kaakkola, 1978). The present results do not give any indication where the opiate receptors, which on stimulation initiate an increase of dopamine synthesis, would be situated.

In the present experiments, morphine, at a high dose and allowed time to act, increased the forma-

tion of 5-HTP. This agrees with several observations that acute morphine administration increases the formation of 5-hydroxytryptamine (5-HT) and the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the brain (Haubrich & Blake, 1973; Yarbrough, Buxbaum & Sanders-Bush, 1973; Goodlet & Sugrue, 1974). β-Endorphin increased the formation of 5-HTP later than that of dopa. The increase occurred in all parts of the brain.

Both naloxone and naltrexone tended to decrease the formation of 5-HTP, so changes in the activity of opiate receptors appear to influence the formation of 5-HT as well as that of dopamine.

Similarly to Goodlet & Sugrue (1974), we found that morphine increased, although slightly, the concentration of cerebral tryptophan at 1 h after administration. We also found that naloxone slightly decreased the cerebral tryptophan concentration. Therefore it is possible that the opiate receptors are involved primarily in the regulation of the transport of tryptophan, the alterations of which secondarily affect the synthesis of 5-HT. However, in view of the relatively slight increase in tryptophan concentration observed after morphine treatment, this is unlikely. Contrary to our findings with morphine, β-endorphin-induced enhancement of the formation of 5-HTP seems to precede the increase in tryptophan concentration, which did not occur until 2 h after β-endorphin.

In addition to tryptophan concentration, morphine also increased the cerebral concentration of tyrosine but clearly later than that of dopa formation. It was most prominent (71%) in the noradrenaline-predominant parts of the brain. β-Endorphin increased tyrosine concentrations in all parts of the brain by 30–60%. The maximum increase in tyrosine concentration occurred at 1·5 h after β-endorphin except in the striatum where, it was at 2 h, and in which the maximum increase of the dopa formation also occurred latest of the brain regions studied. Thus both the β-endorphin- and the morphine-induced increases in dopa formation preceded the increase in tyrosine concentration and they most probably are not connected with the increased dopamine synthesis induced by these compounds.

In spite of minor differences which could be due to different modes of administration, the action of intracerebroventricularly administered β-endorphin on brain monoamine synthesis is remarkably similar to the action of intraperitoneally administered morphine. Moreover, the effect of β-endorphin on dopa formation could be reversed by naloxone. Unfortunately we did not have enough β-endorphin to try to reverse its action on 5-HTP, tyrosine and tryptophan by naloxone. Our observations support the view that β-endorphin exerts its action on monoamine synthesis via opiate receptors.

In conclusion, the present results show that the effects of morphine and β-endorphin and those of naloxone and naltrexone on the synthesis of dopamine and 5-HT are opposite to each other and it is probable that opiate receptors and their endogenous ligands are involved in the regulation of the synthesis of these monoamines.

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