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NALOXONE AFFECTS BOTH PHARMACOKINETICS AND PHARMACODYNAMICS OF MORPHINE

APPLICATION OF DIRECT CORRELATION ANALYSIS

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Abstract—Direct correlation analyses between the distribution of morphine (pharmacokinetics) and the biochemical effects of the drug on monoamine metabolism (pharmacodynamics) are reported for dissected regions of the brain. Determinations of morphine and monoamine-related substances were carried out in the same sample by high performance liquid chromatography with electrochemical detection. Naloxone, an antagonist of morphine, significantly shortened the biological half lives of morphine in both the blood and brain tissue. Such pharmacokinetic behavior appeared to be related to the contractive effect of morphine on the bile duct, and naloxone facilitated the excretion of morphine via this route. In the striatum, significant correlations were observed between the concentrations of the metabolites of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and morphine with a shift to the right in the concentration–response curve on naloxone treatment indicating competitive antagonism. While significant correlations were also observed in this brain region for the metabolites of noradrenaline, 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG), and 5-hydroxytryptamine, 5-hydroxyindoleacetic acid (5-HIAA), a shift to the right did not occur. Significant correlations and shifts were noted for DOPAC, HVA and MOPEG in the hypothalamus. However, no correlation was found between the concentrations of 5-HIAA and morphine in this region. In other regions such as the hippocampus and medulla oblongata, similar correlations and shifts were not observed for MOPEG and 5-HIAA or for DOPAC and HVA. These results suggest that the biological effects of morphine show themselves primarily in the dopaminergic system of the brain, and the noradrenergic system of the hypothalamus.

Morphine is one of the most important drugs for the management of intolerable pain at clinical institutes. This compound is also of interest for its pharmacological and toxicological properties including the physicopathological mechanisms of pain and drug dependence. It has been shown that morphine affects monoaminergic transmission in the central nervous system via its biochemical effects on the dopaminergic [1–4], noradrenergic [5, 6] and serotonergic systems [7–10]. In previous reports, we demonstrated that direct correlation analysis, in which the concentrations of administered drug and monoamine metabolites were simultaneously determined in the same brain sample, represents a simple technique for determining the real potency of the drug and provides a useful approach for biochemical pharmacology [11–13]. Morphine was found by this procedure to affect mainly the dopaminergic system rather than the serotonergic system in mouse whole brain sample [12]. However, the central nervous system displays a multi-functional distribution with different transmitter systems, according to the anatomical region, which interact to modulate the brain function in a balanced manner [14]. This means that whole brain assay is unfortunately unable to reveal the fine effects of a drug on a specific region. Thus, regional assays of the effects of morphine are needed in order to elucidate the precise site of action of this drug.

The site of action of morphine has been confirmed in many reports to be the opiate receptor [15]. Such receptors are distributed unevenly in the central nervous system with the different subtypes [16, 17] having different pharmacological effects [15, 18]. Morphine is known to be a typical agonist for the μ-type opiate receptor. On the other hand, naloxone acts as a competitive antagonist of opiates in both pharmacological [6, 7, 9] and electrophysiological studies [19, 20]. This antagonist has recently been shown to display a specific blocking property on μ receptors [18], which regulate the pain mechanism. In *in vitro* experiments, the concentration–response curve of a drug is known to be shifted to the direction of higher dose if a selective competitive antagonist is present [21]. The aim of the present study was to elucidate the precise site and mode of action of morphine on the metabolism of monoamine transmitters in the brain using direct correlation analysis and the competitive antagonism of naloxone on the neurochemical effects of morphine. During the course of the study, it became apparent that naloxone affected not only the pharmacodynamics but also the pharmacokinetics of morphine.

MATERIALS AND METHODS

Animals. Male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing about 250 g were employed in the present study. The

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animals were housed in a room where the temperature (23 ± 0.5°), humidity (55 ± 5%) and light cycle (12 hr light/12 hr dark with the light turned on at 7:00 a.m.) were controlled for at least 1 week prior to the experiments. They received standard rat chow and water *ad lib.* during this period.

Chemicals. Morphine hydrochloride was purchased from Yoshitomi Pharmaceuticals (Osaka, Japan). Naloxone hydrochloride was a generous donation from Takeda Pharmaceuticals (Osaka). These drugs were dissolved in physiological saline and were always injected intravenously via the tail vein. The chromatographic authentic substances used were all purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). These included noradrenaline (NA*), dopamine (DA), 5-hydroxytryptamine (5-HT), 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA). The internal standard substances, isoproterenol (for the assay of monoamines including morphine and MOPEG) and 3,4-dihydroxyphenylpropionic acid (for the assay of acidic metabolites of monoamines) were obtained from the Sigma Chemical Co. and ICN Pharmaceutical (Plainview, NY, U.S.A.), respectively. All other chemicals employed for extraction and chromatography were obtained from a single source (Wako Pure Chemicals, Osaka) and were used without further purification.

Sample preparation. The blood samples were collected from the carotid artery via an installed catheter at different times after intravenous administration of morphine (10 mg/kg) with or without naloxone (1 mg/kg). The blood was stored in a deep freeze (−80°) without separation until the assay was carried out. In another series of experiments, rats were killed 2 hr after intravenous administration of different doses (0.1–30 mg/kg) of morphine with or without naloxone (1 mg/kg) by microwave irradiation (5 KW, for 1.2 sec). The brain was removed as quickly as possible and placed on an ice-cold plate. A blood sample was then obtained to monitor the concentration of morphine. The brain was dissected into nine regions: the anterior cortex, posterior cortex, amygdala, hippocampus, striatum, hypothalamus, midbrain, ponsmedulla oblongata and cerebellum; according to the procedures of Glowinski and Iversen [22]. Each sample was weighed, wrapped in aluminium foil and stored in a deep freeze.

Extraction of morphine and monoamine-related substances. Extraction for the simultaneous determination of morphine and monoamine-related substances was carried out by a combination of the procedures reported previously [23, 24]. Briefly, the brain sample was transferred to a glass-stoppered tube which contained 0.025 N HCl (the volume differed according to the sample weight) and internal standard substances for chromatography. The sample was homogenized by a Polytron (10,000 rpm, for 10 sec; Kinematica, Luzern, Switzerland). The homogenate was centrifuged for 10 min. The supernatant was transferred to a polyethylene tube for determination of the neutral metabolite of NA, MOPEG. Sulfatase (H-1 type, Sigma Chemical Co.) was added to the tube to hydrolyse the sulfate conjugate and the supernatant was incubated at 37° for 8 hr. The remaining homogenate in the glass-stoppered tube was used for the determination of morphine and monoamine related substances, excluding MOPEG. *n*-Butanol and solid NaCl were added to the homogenate, and the tube was shaken on a reciprocal shaker for 60 min. After centrifugation for 10 min, the supernatant was transferred to another glass-stoppered tube containing *n*-heptane

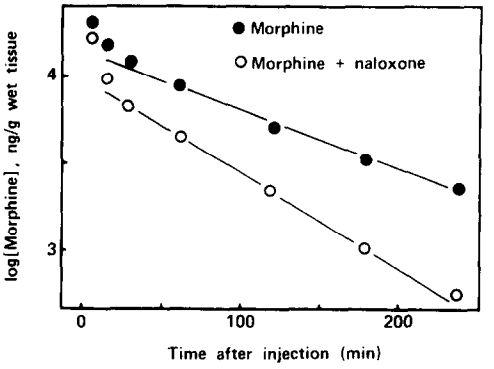

Fig. 1. Effect of naloxone on the elimination of morphine from the blood. Each point represents the mean of six determinations. The correlation coefficients were estimated to be 0.987 and 0.986 for morphine only and morphine plus naloxone, respectively. The biological half lives in the elimination phase were estimated to be 75.8 ± 13.5 and 55.8 ± 5.3 min for morphine only and morphine plus naloxone, respectively.

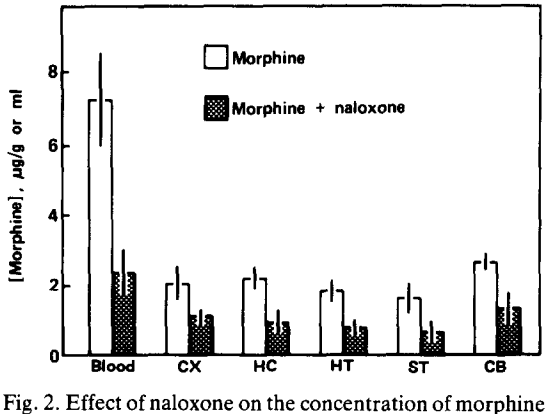

Fig. 2. Effect of naloxone on the concentration of morphine in the blood and brain. Morphine (10 mg/kg) was injected with or without 1 mg/kg of naloxone. The rats were killed 2 hr after the injection. The regional concentration of morphine was determined in the anterior cortex (CX), hippocampus (HC), hypothalamus (HT), striatum (ST) and cerebellum (CB) by high performance liquid chromatography with electrochemical detection. Each column and bar represents the mean ± SD of six determinations.

* Abbreviations: NA, noradrenaline; DA, dopamine, 5-HT, 5-hydroxytryptamine; MOPEG, 3-methoxy-4-hydroxyphenylethylene glycol; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid); 5-HIAA, 5-hydroxyindoleacetic acid.

and 0.025 N HCl. The tube was again shaken on the reciprocal shaker for 10 min and centrifuged for 5 min to separate the aqueous layer which formed the sample for the assay of morphine and monoamines. Tris–HCl buffer (pH 8.3) was added to the organic layer in the tube which was then vortexed for 60 sec and centrifuged briefly. The buffer layer was used as the sample for the determination of acidic metabolites of monoamine transmitters. The blood samples were processed for the determination of morphine according to the procedure used in the case of the brain samples.

Chromatography. Three different chromatographic systems were used in the present study for the determination of (1) monoamine transmitters with morphine, (2) MOPEG and (3) acidic metabolites of monoamines. A liquid chromatographic system (Model 510, Waters Association, MA, U.S.A.) was employed with an automated sample processor (WISP 710B, Waters Association) and a glassy carbon electrochemical detector (ED-100, Eicom, Kyoto, Japan). The analytical column consisted of an Ultrasphere-ODS reversed-phase column (average particle size, 5 μm; 250 × 4.6 mm i.d.; Altex Scientific, Berkeley, CA, U.S.A.). The detector potential was set at 700 (for monoamine and morphine), 750 (for MOPEG) or 600 mV (for acidic metabolites) vs the Ag/AgCl reference electrode. Two different mobile phases were used: 0.05 M sodium citrate/citric acid buffer (pH 4.0) containing 1% tetrahydrofuran for monoamines with morphine and MOPEG, and 0.075 M sodium citrate/citric acid buffer (pH 3.5) containing 1% tetrahydrofuran, 10% methanol and 13% acetic acid for acidic metabolites (see Refs 23 and 24 for detailed chromatographic conditions).

Protein binding of morphine in vitro. The protein binding assay was carried out *in vitro* according to the following procedure. Serum was obtained from naive animals and 1 mL was transferred to a polyethylene tube. Morphine (10 μg) was then added, with or without 10 or 1 μg of naloxone. The tube was incubated at 37° for 10 min. The serum was filtered on an ultrafiltration membrane (UFM-20, Amicon, Tokyo, Japan). The filtrate was employed as the sample for estimating the protein-free drug after adding the internal standard. The concentration of total drug (free + protein bound) was determined by direct injection of the serum sample with deproteinization by perchloric acid.

Elimination of bromsulphalein (BSP). The animals were injected intravenously with 20 mg/kg of BSP. Blood samples were collected from the catheter installed in the carotid artery 2, 4, 6 and 8 min after injection. After separation, 100 μL of plasma were diluted 5 to 40 times and 0.1 N NaOH was added. BSP was determined colorimetrically under 565 nm.

RESULTS

Effect of naloxone on elimination of morphine in blood

The concentration of morphine was measured in the blood after 10 mg/kg of the drug had been injected intravenously. A simulation curve of the concentration was obtained by the non-linear least squares fitting method (Fig. 1). A two compartment model provided the best fit for the simulation, in which two apparent phases, distribution and elimination, could be distinguished. The biological half life of morphine in the elimination phase was estimated to be 76 ± 9 min from this simulation curve, using a computer. When naloxone (1 mg/kg) was injected concurrently, the biological half life of the drug was shortened significantly (56 ± 5 min; $P < 0.01$, Student's *t*-test).

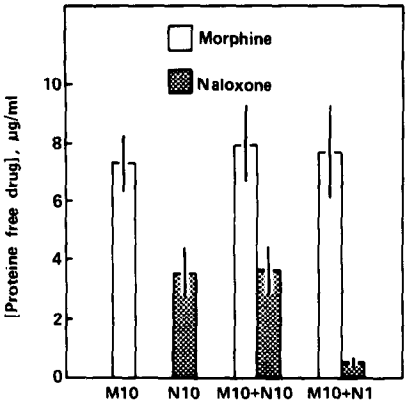

Fig. 3. Effect of naloxone on the binding of morphine to plasma protein. Morphine (10 μg) and/or naloxone (10 or 1 μg) were incubated in plasma for 10 min. The plasma was then ultrafiltrated on a membrane (UFM-20) which allowed substances with a molecular weight of less than 20,000 to pass. The resultant filtrant included protein-free drugs, which were measured by high performance liquid chromatography with electrochemical detection. Each column and bar represents the mean ± SD of 6 determinations.

Effect of naloxone on intracerebral concentration of morphine

The concentration of morphine in the dissected brain regions and blood was measured 2 hr after intravenous injection of the drug with or without naloxone (Fig. 2). The concentration of morphine in the blood decreased from 7.1 to 2.2 μg/mL following concurrent administration of naloxone (see also Fig. 1). The decrease was about 35% compared to the level in animals received the same dose of morphine without naloxone. The intracerebral distribution of morphine was not significantly different among the regions, while the highest concentration was observed in the cerebellum and the lowest, in the striatum (Fig. 2). The intracerebral concentrations of morphine were also influenced by naloxone. The decreases were in the range of 40 to 60% as compared to the levels in animals which did not receive concurrent administration of naloxone. Such decreases were observed when more than 1 mg/kg of morphine was administered with 1 mg/kg of naloxone.

Effects of naloxone on protein binding of morphine

Both morphine and naloxone could be detected, without any significant interference from biogenic substances, by high performance liquid chromatography with electrochemical detection (chroma-

Table 1. Effects of morphine and naloxone on the elimination of bromsulphalein (BSP) from rat blood

Treatment	K_{el}/min
Control	0.27 ± 0.02
Morphine 10 mg/kg	0.16 ± 0.02*
Naloxone 1 mg/kg	0.25 ± 0.02
Morphine 10 mg/kg + naloxone 1 mg/kg	0.30 ± 0.05

The animals were first injected intravenously with morphine and/or naloxone. BSP (20 mg/kg) was injected intravenously 30 min after drug administration. Blood samples were collected 2, 4, 6 and 8 min after the BSP injection from a catheter installed in the carotid artery. Following separation, 100 μL of plasma were diluted 5 to 40 times and 0.1 N NaOH was added. The BSP was determined colorimetrically at 565 nm.

K_{el}, elimination constant of BSP.

* Significantly ($P < 0.01$) different from the control.

togram not shown). The concentration of protein free morphine in the ultrafiltrate from the membrane, which allowed substances with a molecular weight of less than 20,000 to pass, was 7.5 μg/mL (Fig. 3). Since the concentration added initially was 10 μg/mL, the protein binding ratio was 25%. On the other hand, the protein binding of naloxone was estimated to be 48%. A combination of morphine and naloxone (10 μg/mL each) did not influence the protein binding ratio of either substance. Addition of 1 μg/mL of naloxone also did not affect the binding of morphine to the serum protein (Fig. 3).

Effect of morphine on elimination of BSP from blood

The elimination constant (K_{el}) of BSP in the control animals was 0.27 ± 0.02 min (Table 1). Morphine significantly ($P < 0.01$) reduced this constant to 0.16 ± 0.02 min^{-1}. Although 1 mg/kg of naloxone did not itself affect the elimination constant, this drug brought about recovery of the reduced constant when injected with 10 mg/kg of morphine.

Concentration-dependent effect of morphine on monoamine metabolism

Different doses (0.1–30 mg/kg) of morphine were injected intravenously into rats 2 hr prior to microwave irradiation and the concentrations of the drug and monoamine metabolites were measured in the same samples of brain tissue. These measurements provided data for direct correlation analysis between the concentrations of morphine and monoamine metabolites in the dissected brain regions. In the hypothalamus, the concentrations of DOPAC increased linearly depending on the concentration of morphine (Fig. 4). The regression line between the concentrations of morphine and DOPAC showed a significant correlation ($r = 0.82$, $N = 24$, $P < 0.001$). A significant correlation was also observed when morphine was administered concurrently with naloxone ($r = 0.60$, $N = 24$, $P < 0.01$; Table 2). Co-variance analysis was carried out on these regression lines and a significant difference was observed in the height of the intercepts but not in the slopes (Table 2). This meant that the two regression lines were parallel with

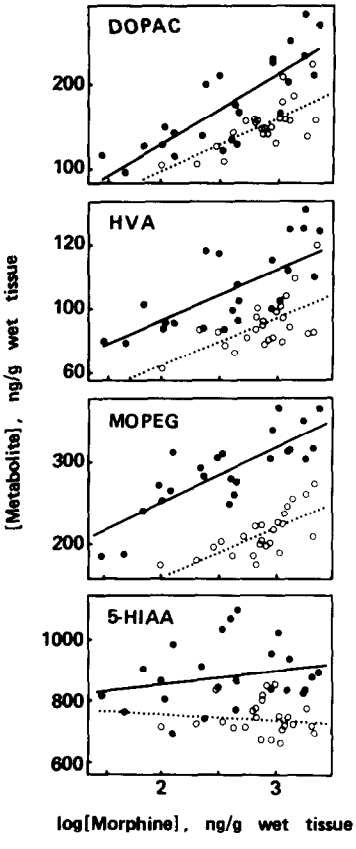

Fig. 4. Correlation analysis between the concentrations of morphine and monoamine metabolites in the hypothalamus. Different doses of morphine were injected intravenously with (○) or without (●) 1 mg/kg of naloxone. The rats were killed 2 hr after the injection and the concentrations of both morphine and monoamine metabolites were determined in the same samples using high performance liquid chromatography with electrochemical detection. See Table 2 for the statistical values of the regression lines.

Table 2. Statistical results for direct correlation analysis between the concentrations of morphine and monoamine metabolites in the hypothalamus

	DOPAC	HVA	MOPEG	5-HIAA
Regression lines				
Morphine alone				
$Y = aX + b$	$Y = 74x + 22$	$Y = 30X + 32$	$Y = 66X \frac{1}{2} 115$	$Y = 41X + 666$
r	0.82	0.67	0.80	0.24
P	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
Morphine + naloxone				
$Y = aX + b$	$Y = 59X - 23$	$Y = 28X + 9$	$Y = 59X + 37$	$Y = -15X + 688$
r	0.60	0.58	0.72	−0.09
P	$P < 0.01$	$P < 0.01$	$P < 0.001$	NS
Co-variance analysis				
r	NS	NS	NS	NS
Slope	NS	NS	NS	$P < 0.01$
Height	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS

NS, not significant.

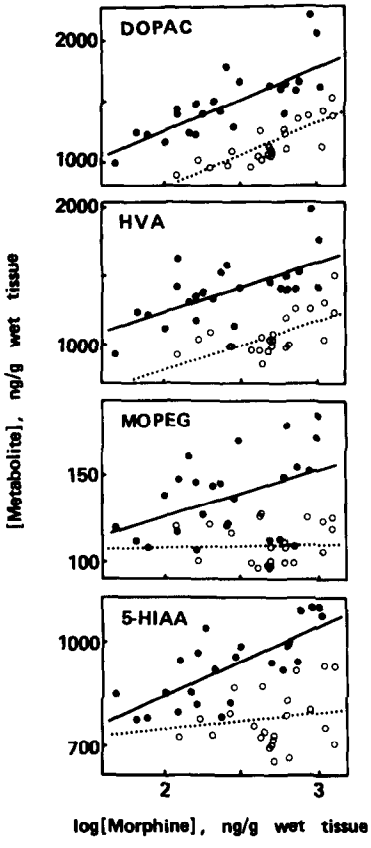

Fig. 5. Correlation analysis between the concentrations of morphine and monoamine metabolites in the striatum. Symbols as in Fig. 4. See Table 3 for the statistical values of the regression lines.

a shift to the right in the case of concurrent administration. Similar results were obtained from analyses of the concentrations of morphine and MOPEG and morphine and HVA. On the other hand, no significant correlation coefficient was found for the regression line between the concentrations of morphine and 5-HIAA when morphine was injected either with or without naloxone.

In the striatum, significant correlations were noted between the concentrations of morphine and DOPAC, HVA and 5-HIAA, but not between the drug and MOPEG in the case where morphine was administered without naloxone (Fig. 5 and Table 3). When the animals were administered concurrently with 1 mg/kg of naloxone, significant correlations were also observed in the regression lines between the concentrations of morphine and both the metabolites of DA, i.e. DOPAC and HVA, but not between morphine and 5-HIAA (Table 3). Parallel shifts of the regression lines were noted for DOPAC and HVA when naloxone was concurrently administered. Similar analyses were carried out for the other brain regions, and the results are summarized in Table 4.

DISCUSSION

Direct correlation analysis, in which the concentration of a drug and the change in concentration of monoamine metabolites induced by the drug are determined simultaneously, has been shown to be a simple and valuable technique for comparing the influence of drugs on monoamine metabolism [11–13]. In the present study, the effect of morphine in dissected brain regions was analysed by this technique in an attempt to elucidate its site of action. Naloxone is a specific antagonist of morphine and its mode of action involves the competitive blocking of opiate receptors (see Refs 15 and 18 for review). This antagonist has been reported to bring about recovery to the original level of the concentration of monoamine metabolites after the increase induced by morphine [9]. Since few investigations of the pharmacokinetic influence of naloxone on morphine have been carried out, the present study examined also the effect of naloxone on the pharmacokinetic fate of morphine in rats.

Table 3. Statistical results for direct correlation analysis between the concentrations of morphine and monoamine metabolites in the striatum

	DOPAC	HVA	MOPEG	5-HIAA
Regression lines				
Morphine alone				
$Y = aX + b$	$Y = 550X + 137$	$Y = 334X + 133$	$Y = 27X + 80$	$Y = 202X + 430$
r	0.76	0.59	0.39	0.72
P	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$
Morphine + naloxone				
$Y = aX + b$	$Y = 543X - 327$	$Y = 338X - 528$	$Y = 2X + 113$	$Y = 42X + 655$
r	0.78	0.63	0.05	0.12
P	$P < 0.001$	$P < 0.001$	NS	NS
Co-variance analysis				
r	NS	NS	NS	$P < 0.01$
Slope	NS	NS	NS	$P < 0.01$
Height	$P < 0.01$	$P < 0.01$	NS	NS

NS, not significant.

In the blood, the pharmacokinetics of morphine were found to be affected by concurrent administration of naloxone which shortened significantly the biological half life of morphine in the elimination phase. Morphine was distributed evenly in the brain with no significant difference between regions. Naloxone decreased significantly the intracerebral concentration of morphine. The rate of decrease was almost the same in each region, about 50%. Binding of a drug to plasma proteins limits the penetration of the drug to the locus of action and limits the glomerular filtration of the drug, which influences the concentration of the drug in tissues [25]. The present results demonstrate that naloxone did not affect the protein binding of morphine. It is reasonable to infer, therefore, that the decrease in the intracerebral concentration of morphine is the result of a decrease in concentration in the circulatory system without any change in the glomerular filtration rate. In the case where 1 mg/kg of morphine was injected, the same dose of naloxone did not affect the concentration of morphine in the blood. However, naloxone did decrease the concentration of morphine injected at dose levels of over 5 mg/kg. The decreases were similar in groups administered with different doses of the drug of between 5 and 30 mg/kg. Morphine is known to be excreted into the bile duct [15]. This drug is also known to exert a contractive effect on smooth muscle which tends to restrict its excretion into the small intestine via the bile duct [15]. This was confirmed by the observation reported here that the elimination of BSP was prolonged by morphine administration and was returned to normal by concurrent administration of the narcotic antagonist. Naloxone caused a rapid excretion of morphine because of its antagonistic effect towards the contraction and this resulted in a decreased concentration of the drug in the body. At a dose level of 1 mg/kg, morphine probably does not exert a significant effect on the smooth muscle of the bile duct, and thus, naloxone had no apparent effect on the concentration of morphine.

Previous reports concerning the effect of naloxone on the metabolism of monoamine-related substances have suggested that it is a function of the administered dosage. However, the present pharmacokinetic findings demonstrate that even if the same dose of morphine was injected, the intracerebral concentration of the drug decreased by one half in comparison with the case where naloxone was administered concurrently. The pharmacodynamic effect of a drug appears to be a function of the concentration of the drug at the site of action. This implies that a simple comparison between the effects of naloxone on the change in concentration of different monoamine-related substances induced by morphine does not reflect the true pharmacodynamics of the drug. Direct correlation analysis is thus required in order to eludicate the precise influence of the narcotic antagonist on the change in intracerebral concentrations of monoamine-related substances induced by morphine.

Morphine is known to affect the opiate receptor [15], which may interact with the dopaminergic receptor [26–28]. Morphine increased the concentrations of DA metabolites in a dose-dependent manner in the striatum, while it did not affect the concentration of DA itself. These results imply that the drug may increase the turnover rate of DA which might be due, at least in part, to augmented release of the transmitter from the nerve terminals. Morphine was found also to increase the release of 5-HT but not that of NA from this region, as suggested by the fact that a significant correlation was observed between the concentrations of morphine and 5-HIAA on the concentration–response curves. In the pharmacological sense, competitive antagonism shifts the log concentration–response curve to the right [21]. In the present study, the changes in concentration of DOPAC and HVA, but not of 5-HIAA, were shifted to the right on the concentration–response curves by concurrent administration of naloxone. This suggested that, in the striatum, naloxone competitively antagonized the effect of morphine on the dopaminergic system, but not that on either the serotonergic or the noradrenergic system. It is well established that naloxone acts as an antagonist of μ-type opiate

Table 4. Summary of direct correlation analyses between the concentrations of morphine and monoamine metabolites in dissected brain regions

Region	Noradrenaline	Dopaminergic	Serotonergic
Anterior cortex	Significant	Significant	NS
Posterior cortex	NS	Significant	NS
Amygdala	NS	Significant	NS
Hippocampus	NS	NS	NS
Hypothalamus	Significant	Significant	NS
Striatum	NS	Significant	NS
Midbrain	NS	Significant	NS
Pons-medulla	NS	NS	NS
Cerebellum	NS	NS	NS

The analyses were performed in all regions listed below according to the procedures shown in Tables 2 and 3. The term "significant" was assigned when the following criteria were satisfied: (1) the regression coefficients were statistically significant ($P < 0.01$) for both morphine alone and morphine plus naloxone, and in the covariance analysis, (2) the regression coefficient and (3) the slopes of the two regression lines were not significantly different and (4) the intercepts of the lines were significantly different. Significant sites can be concluded to be those where morphine acts directly, via naloxone-preferential opiate receptors, on the monoaminergic system.

NS, not significant.

receptors which mediate the release of monoamine transmitters from nerve terminals [29]. However, since the concentration of morphine (10 ng/g wet tissue at the site of action) required to produce the present biochemical effects was far higher than that required for receptor binding assay, it was impossible to decide the receptor subtype. It seems reasonable to suppose that the effect of morphine on the dopaminergic system may be meditated by "naloxone-preferential" receptors rather than μ receptors. If naloxone blocks the naloxone-preferential opiate receptors, the release of DA from the nerve terminals will be attenuated resulting in a decrease in the extraneuronal metabolite of this transmitter, HVA [30, 31]. The present data, however, revealed an increase in HVA as well as in the intraneuronal metabolite, DOPAC. In a recent report, we applied direct correlation analysis to phenothiazine derivatives [13]. In these experiments, four derivatives were compared regarding the slope of the regression curve between concentrations of phenothiazine and monoamine metabolites. The ratio of the slope for HVA to DOPAC was larger in those drugs possessing a strong receptor blocking property. This indicated that HVA might reflect the extraneuronal process of DA metabolism. In the experiments reported here, this ratio showed a value of 334/550 (see Table 2) which was markedly smaller than that for competitively antagonistic phenothiazines [13]. These findings suggest the possibility that morphine may act on the presynaptic catabolism of DA as well as on the extraneuronal process. Similar effects of naloxone were observed in the anterior and posterior cortices, amygdala, hypothalamus and midbrain, but not in the hippocampus, pons-medulla or cerebellum. These findings reflect the distribution of naloxone-preferential opiate receptors [16] and dopaminergic nerve terminals [32, 33].

No significant correlation was noted between the concentrations of morphine and MOPEG in the striatum when the drug was injected either with or without the competitive antagonist naloxone. This implied that morphine did not directly affect the noradrenergic system in the striatum and might be consistent with the fact that noradrenergic innervation has not been documented in this structure [33]. On the other hand, MOPEG was increased in a concentration-dependent manner in the hypothalamus following the administration of morphine. The same phenomenon was also observed when the drug was administered with naloxone. The two regression lines were parallel to each other, with a shift to the right when the antagonist was administered concurrently. This indicated that naloxone competitively antagonized the effect of morphine on the noradrenergic system of this region. A similar effect was observed in the anterior cortex but not in the other regions examined. Thus, in the two regions which revealed a significant interaction between morphine and naloxone, the naloxone-preferential opiate receptors may couple with the noradrenergic system. There was no significant interaction between morphine and naloxone on the serotonergic system. The naloxone-preferential receptors could therefore not mediate the function of the serotonergic system in the brain.

The present chromatographic technique provides a means for undertaking simultaneous measurements of morphine and substances related to monoamine metabolism and permits comparisons to be made between biochemical effects of drugs expressed as a function of concentration at the site of action, similarly to *in vitro* techniques. The present data suggest that morphine acts mainly on the dopaminergic system in the brain, interacting with naloxone-preferential (probably μ) receptors. Its action is also induced through the noradrenergic system in some regions of the brain. However, no biochemical action of morphine is apparent in the central serotonergic system.

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