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Modification of the Development of Acute Opiate Tolerance by Increased Dopamine Receptor Sensitivity[1]

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ABSTRACT

Earlier studies have suggested that the acute administration of an opiate can result in the development of supersensitive dopamine receptors. The present study was undertaken to determine whether the supersensitive dopamine receptors can modify the development of opiate tolerance and dependence. Administration of morphine (100 mg/kg s.c.) 6 or 24 hr before apomorphine (i.p.) potentiated apomorphine-induced climbing behavior in mice. Administration of levorphanol (12 mg/kg s.c.) 3 or 6 hr, but not 24 hr, before apomorphine also potentiated apomorphine-induced climbing behavior. Coadministration of 5 mEq/kg of LiCl with morphine or levorphanol attenuated the increased sensitivity developed to apomorphine after either opiate. Acute tolerance and dependence was induced by administration of 100 mg/kg of morphine or 12 mg/kg of levorphanol. Lithium enhanced the development of acute tolerance when coadministered with morphine 3, 6 or 24 hr before test doses of morphine, or with levorphanol 3 hr before test doses of levorphanol. Administration of apomorphine 5 min before naloxone significantly decreased the naloxone ED_{50} for inducing withdrawal jumping in mice that had been pretreated with morphine or levorphanol. Although coadministration of lithium with morphine or levorphanol had no significant effect on naloxone-induced withdrawal jumping, it attenuated the ability of apomorphine to decrease naloxone ED_{50}. Morphine (100 mg/kg s.c.) increased the number of whole brain [^{3}H]spiroperidol binding sites 3 and 6 hr after administration of morphine. This increase was no longer present 24 hr after morphine administration. Levorphanol (12 mg/kg s.c.) also increased the number of binding sites 3 hr after administration. Coadministration of lithium with morphine attenuated the increase in [^{3}H]spiroperidol binding sites. These results suggest that acute administration of an opiate can increase the sensitivity of dopamine receptors. This increase in sensitivity can then modify the degree of analgesic tolerance, and possibly dependence, which develops after opiate administration.

A number of anatomical studies have established an overlap of dopaminergic and enkephalinergic neurons in several brain regions including the ventral tegmental area, substantia nigra, nucleus accumbens and caudate nucleus (Fallon and Moore, 1978; Moore and Bloom, 1978; Miller and Cuatrecasas, 1979; Rossier and Bloom, 1979). Such an overlap of these two systems would suggest that they interact with one another. In support of this suggestion it has been found that opiates can influence the synthesis (Urwyler and Tabakoff, 1981; De Simoni *et al.*, 1982; Ishikawa *et al.*, 1983; Spampinato *et al.*, 1984), turnover (Loh *et al.*, 1973; Alper *et al.*, 1980; Guaza *et al.*, 1980) and release of DA from dopaminergic neurons (Loh *et al.*, 1976; Wood *et al.*, 1980; Chesselet *et al.*, 1981a,b). Furthermore, chronic treatment of rats with haloperidol has been reported to elevate the levels of methionine enkephalin in the striatum (Hong *et al.*, 1985). These findings suggest that DA might modulate enkephalinergic neurons, and that opiates might modulate dopaminergic neurons.

Further evidence that opiates can modulate dopaminergic systems derives from results obtained from chronic morphine treatment of rats. Such treatment has been found to increase the sensitivity of rats to the stereotypic behavior inducing effects of the DA agonist apomorphine (Puri and Lal, 1973; Eidelberg and Erspamer, 1975; Ritzmann *et al.*, 1979; Tye *et al.*, 1979; Bhargava, 1980). Increases in the affinity of the DA receptor for the DA antagonist spiroperidol has also been reported to occur concomitantly with the increased sensitivity to apomorphine (Ritzmann *et al.*, 1979; Bhargava, 1980). An operant behavior study also indicated the development of supersensitive DA receptors in rats treated chronically with morphine (Christie and Overstreet, 1979), although these investigators found no alterations in DA receptor binding parameters at the time of the behavioral supersensitivity. The results of these studies provide behavioral and biochemical evidence that chronic opiate treatment can alter dopaminergic sensitivity. None of these studies explored the possible effect of a single dose of an opiate on DA receptor sensitivity.

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ABBREVIATIONS: DA, dopamine; PLG, prolyl-leucyl-glycinamide.

Little information is available concerning the effect a single administration of an opiate might have on DA receptor sensitivity. A single administration of morphine has been reported to increase the sensitivity of mice to the stereotypic effects of apomorphine (De la Baume *et al.*, 1979). Our earlier findings suggest that single low doses of morphine or levorphanol can induce the development of supersensitive DA receptors in mice (Martin and Takemori, 1985). Previous investigations, all of which were performed on rats, suggest that blockade of the development of supersensitive DA receptors which follows chronic morphine treatment can result in alterations of analgesic tolerance (Ritzmann *et al.*, 1979; Bhargava, 1980). The present study was undertaken, therefore, to determine if a single administration of morphine or levorphanol, at doses which result in the development of acute tolerance and dependence, can induce the development of supersensitive DA receptors. Furthermore, it was determined whether the blockade of the development of the supersensitive DA receptors could alter the degree of tolerance and/or dependence which developed to the opiate. Lithium was used to block the development of the supersensitive DA receptors based on our earlier observations that lithium administered concurrently with low doses of morphine or levorphanol could inhibit the development of DA receptor supersensitivity (Martin and Takemori, 1985). The results of this study suggest that the development of supersensitive DA receptors after a single administration of an opiate can modify the degree of acute tolerance and dependence which develops in response to the opiate administration.

Materials and Methods

Animals. Random bred male Swiss-Webster mice (Biolab, White Bear, MN) weighing between 20 and 25 g were used in all experiments. All animals were supplied food and water *ad libitum*. They were housed for at least 1 day before experimentation. Each animal was used only once.

Analgesic assays and assessment of tolerance. Analgesia was assessed using the tail-flick assay of D'Amour and Smith (1941) as modified by Tulunay and Takemori (1974), and the hot-plate assay (Eddy and Leimbach, 1953). Control latencies were determined before drug administration. Test latencies were determined 30 min after the administration of various test doses of morphine or levorphanol. An animal was considered analgesic if its test latency time was greater than its control latency time by more than 3 times the S.D. of the mean control latencies of that mouse's group. Thus, the analgesic responses to morphine or levorphanol were made quantal. There were at least eight animals per group, and the analgesic response of three groups were used to determine an ED_{50} for either morphine or levorphanol. The ED_{50} value and its 95% CL were determined by use of a computer program of the parallel line assay of Finney (1964).

Acute tolerance was induced by treating mice with a single 100-mg/kg dose of morphine (Yano and Takemori, 1977) or a 12-mg/kg dose of levorphanol (Contreras and Takemori, 1984). Three, 6 or 24 hr later the mice were tested to ensure that their latencies had returned to control values. In some experiments 5 mEq/kg of LiCl was coadministered with saline, 100 mg/kg of morphine or 12 mg/kg of levorphanol 3, 6 or 24 hr before test doses of opiate and subsequent analgesic testing. Thirty minutes after a test dose of opiate the mice were again tested for analgesia. Tolerance was considered to be present when there was a significant difference between the experimental ED_{50} of the opiate and the control ED_{50} of the opiate. A significant difference occurred when the control ED_{50} of the opiate was outside the 95% CL of the experimental ED_{50} of the opiate and the experimental ED_{50} of the opiate was outside the 95% CL of the control ED_{50} of the opiate.

Assessment of physical dependence. Acute dependence was induced by administration of 100 mg/kg of morphine or 12 mg/kg of levorphanol. Three or 6 hr later the mice were administered naloxone which induces a withdrawal jumping in dependent mice. This jumping syndrome has proven to be a reliable indicator of precipitated withdrawal in mice (Way *et al.*, 1969). Mice were placed into 30 cm × 30 cm Plexiglas cylinders immediately after the injection of naloxone to determine if a particular dose of naloxone could induce jumping. The number of vertical jumps were counted for each mouse during the next 15 min. A mouse was considered to show a positive response if it jumped five or more times during the 15-min period (Huang *et al.*, 1978). The ED_{50} of naloxone was estimated using the up-and-down method of Dixon (1965) using five to six mice for each estimate. The results of at least five determinations are reported as the mean ± S.E.

The effect of lithium on the development of acute dependence was determined in mice administered 100 mg/kg of morphine 6 hr before naloxone, and in mice administered 12 mg/kg of levorphanol 3 hr before naloxone. Some animals also received lithium which was coadministered with morphine or levorphanol. In other experiments, apomorphine was administered to the morphine- or levorphanol-treated animals 5 min before the administration of naloxone. This 5-min period was allowed for the distribution of apomorphine to the brain of the animal before the administration of naloxone. Thus, the effect of naloxone could be measured at the time apomorphine was exerting its effect. In another set of experiments, lithium was administered 10 min before naloxone (and thus 5 min before apomorphine). This 10-min period was allowed for some distribution of lithium to the brain. The distribution of lithium is rather rapid and significant levels are measurable within 15 min of administration (Schou, 1958; Mukherjee *et al.*, 1976).

Assessment of apomorphine-induced climbing behavior. DA receptor sensitivity was determined in the whole animal by measuring apomorphine-induced climbing behavior (Protais *et al.*, 1976). Mice were treated with saline, 100 mg/kg of morphine or 12 mg/kg of levorphanol 3, 6 or 24 hr before administration of apomorphine. In some experiments 5 mEq/kg of LiCl was coadministered with the saline, morphine or levorphanol. Immediately after administration of apomorphine the mice were placed into climbing cages built to the specifications described by Protais and co-workers (1976). Climbing behavior was scored 10 and 20 min after the administration of apomorphine using the scoring system: four paws on the floor (0), forefeet on the bars or four paws on the bars for only a few sec (1) and four paws on the bars for 1 min (2). The two scores were averaged for each mouse and then expressed as a percentage of a score of 2. At least 10 mice were used for each dose of apomorphine. An ED_{50} value for apomorphine was determined from three different doses of apomorphine. The ED_{50} values and 95% CL were determined for the DA agonists using the computer program for the parallel line assay of Finney (1964). Statistical differences between groups were determined as described previously for analgesic ED_{50} values and their 95% confidence intervals.

Determination of whole brain levels of morphine. Whole brain levels of morphine were measured using the method of Sprague and Takemori (1979). Recovery of added morphine from brain homogenate was 94.2 ± 1.2%. Whole brain levels of morphine were determined 3 hr after administration of 100 mg/kg of morphine (100 μCi/kg) and 30 min after 10 or 5 mg/kg of morphine (200 μCi/kg). Lithium was coadministered with the 100-mg/kg dose of morphine or 3 hr before administration of 10 mg/kg of morphine. In other experiments lithium and 100 mg/kg of morphine were coadministered 3 hr before administration of 30 or 15 mg/kg of morphine (100 μCi/kg).

DA receptor binding. DA receptor binding was performed by utilizing the method described by Burt *et al.* (1976). Mice were sacrificed and their brains removed 3, 6 and 24 hr after treatment with 100 mg/kg of morphine or 3 hr after 12 mg/kg of levorphanol. Some of the animals were coadministered lithium with the morphine. Each brain was homogenized, after removal of the cerebellum, in 40 volumes of ice-cold 50 mM Tris/HCl buffer, pH 7.7, containing 0.1 mM EDTA using a Brinkmann Polytron Homogenizer set at 5.0 for 15 sec. The homogenate was then centrifuged twice at 50,000 × *g* for 10 min using

a Beckman L8-70 Ultracentrifuge. The supernatant was discarded and the pellet was resuspended in fresh buffer between centrifugations. The final pellet was resuspended in 100 volumes of ice-cold 50 mM Tris/HCl buffer, pH 7.1, containing (millimolar): NaCl, 120; KCl, 5; $CaCl_2$, 2; and $MgCl_2$, 1. The homogenate was then incubated at 37°C for 5 min before being frozen.

Total binding was determined by incubating 1 ml of brain homogenate with [^{3}H]spiroperidol (final concentration ranged from 0.30 to 8.0 nM). Nonspecific binding was determined in the presence of 1.0 μM unlabeled (+)-butaclamol (Research Biochemicals, Inc., Wayland, MA). Incubation tubes were prepared in duplicate and incubated at 37°C for 20 min. The content of each tube was filtered rapidly through a Whatman GF/C filter with three 5-ml washes with ice-cold buffer (50 mM Tris/HCl, pH 7.1). Filters were placed into liquid scintillation vials containing 10 ml of Aquasol (New England Nuclear, Boston, MA). Radioactivity was determined by liquid scintillation spectrometry. Specific binding was defined as total binding minus nonspecific binding. Protein concentrations were estimated using the method of Lowry *et al.* (1951). Scatchard plots were analyzed using least-squares linear regression to determine K_d and maximum binding values for at least five individual brains. Significance was determined using analysis of variance. Significance between groups was determined by using least-significant difference.

Drugs. Morphine sulfate (Merck and Company, Inc., Rahway, NJ), levorphanol tartrate (Hoffmann-La Roche, Inc., Nutley, NJ), naloxone hydrochloride (Endo Laboratories, Garden City, NJ) and apomorphine hydrochloride (Merck and Company, Inc.) were dissolved in saline. Apomorphine was prepared on the day of the experiment. All drug dosages are expressed as the salt. Naloxone, apomorphine and LiCl (J. T. Baker Chemical Co., Phillipsburg, NJ) were administered i.p. Morphine and levorphanol were administered s.c. All drugs were administered in a volume of 10 ml/kg.

[^{3}H]Morphine (Amersham/Searle, Des Plaines, IL; specific activity, 24 Ci/mmol) was used to determine whole-brain levels of morphine. [^{3}H]Spiroperidol (New England Nuclear; specific activity, 24.5 Ci/mmol) was used to determine DA receptor binding.

Results

Effect of morphine or levorphanol on apomorphine-induced climbing behavior. Morphine or levorphanol treatment caused a potentiation of apomorphine-induced climbing behavior as seen by a significant decrease in the apomorphine ED_{50} (table 1) which results from a parallel leftward shift of the apomorphine dose-response curve. This potentiation was evident 3 hr after 12 mg/kg of levorphanol, but was not evident until 6 hr after 100 mg/kg of morphine. Although the apomorphine ED_{50} had returned to control levels by 24 hr after levorphanol administration, it was still reduced significantly 24 hr after morphine. The concurrent administration of LiCl with morphine or levorphanol prevented the development of the increased sensitivity to apomorphine 6 and 24 hr after morphine and 6 hr after levorphanol (table 1).

Effect of lithium on the development of acute tolerance to morphine or levorphanol. A single administration of 100 mg/kg of morphine 3 hr before a test dose of morphine resulted in the development of tolerance which was detected as a parallel rightward shift of the dose-response curve of morphine in the tail-flick assay. A similar shift of the dose-response curve was also observed in the hot-plate assay. These shifts resulted in approximately 3-fold increases in the morphine ED_{50} determined from both analgesic assays (table 2). Coadministration of 5 mEq/kg of LiCl with the 100 mg/kg of morphine resulted in an even further parallel shift of the morphine dose-response curve to the right. This resulted in a 6-fold increase in the morphine ED_{50} over the control morphine ED_{50}, and a 2-fold increase of the morphine ED_{50} over that which resulted after treatment with 100 mg/kg of morphine (table 2). A similar degree of tolerance to morphine was observed 6 hr after treatment with 100 mg/kg of morphine. Tolerance, although much less, was observed as long as 24 hr after the single 100-mg/kg dose of morphine. Lithium coadministered with the 100-mg/kg

TABLE 1

Potentiation by opiates of apomorphine-induced climbing behavior

Treatment	Apomorphine ED_{50}[a] (95% CL)
	mg/kg i.p.
3 hr	
Saline + saline[b]	2.2 (1.9–2.6)
Saline + 100 mg/kg of morphine[b]	2.1 (1.8–2.4)
Saline + 12 mg/kg of levorphanol[b]	1.6 (1.4–1.8)*
6 hr	
Saline + saline[b]	2.1 (1.8–2.6)
5 mEq/kg of LiCl + saline[b]	2.1 (1.7–2.5)
Saline + 100 mg/kg of morphine[b]	1.7 (1.5–1.9)*
5 mEq/kg of LiCl + 100 mg/kg of morphine[b]	2.1 (1.8–2.4)
Saline + 12 mg/kg of levorphanol[b]	1.6 (1.3–1.9)*
5 mEq/kg of LiCl + 12 mg/kg of levorphanol[b]	2.2 (1.8–2.6)
24 hr	
Saline + saline[b]	2.3 (1.9–2.7)
5 mEq/kg of LiCl + saline[b]	2.1 (1.8–2.5)
Saline + 100 mg/kg of morphine[b]	1.7 (1.5–1.9)*
5 mEq/kg of LiCl + 100 mg/kg of morphine[b]	2.0 (1.6–2.5)
Saline + 12 mg/kg of levorphanol[b]	2.1 (1.8–2.5)

[a] At least 35 animals were used to construct each dose-response curve from which apomorphine ED_{50} values were determined.
[b] Coadministered at time indicated before apomorphine.
* Significantly different from saline- or lithium-treated controls ($P < .05$).

TABLE 2

Effect of lithium on development of tolerance to 100 mg/kg of morphine

Treatment[a]	Test Morphine ED_{50} (95% CL)[b]	
	Tail flick	Hot plate
	mg/kg s.c.	
3 hr before test morphine		
Saline + saline	4.4 (3.8–5.2)	5.2 (3.7–7.3)
5 mEq/kg of LiCl + saline	4.8 (4.2–5.7)	4.8 (3.4–6.7)
Saline + 100 mg/kg of morphine	15.1 (13.0–17.6)*	17.7 (12.8–25.3)*
5 mEq/kg of LiCl + 100 mg/kg of morphine	34.0 (29.2–39.6)*,†	27.3 (19.3–37.9)*,†
6 hr before test morphine		
Saline + saline	5.0 (4.3–5.9)	5.0 (3.9–6.4)
5 mEq/kg of LiCl + saline	4.5 (3.9–5.3)	4.5 (3.5–5.7)
Saline + 100 mg/kg of morphine	16.1 (13.8–18.9)*	15.6 (12.3–19.9)*
5 mEq/kg of LiCl + 100 mg/kg of morphine	24.2 (20.6–28.4)*,†	28.6 (22.4–36.3)*,†
24 hr before test morphine		
Saline + saline	5.0 (4.3–5.8)	4.5 (4.1–5.0)
5 mEq/kg of LiCl + saline	5.3 (4.6–6.1)	5.0 (4.5–5.5)
Saline + 100 mg/kg of morphine	7.1 (6.1–8.2)*	7.5 (6.8–8.3)*
5 mEq/kg of LiCl + 100 mg/kg of morphine	10.8 (9.4–12.5)*,†	9.8 (8.9–10.8)*,†

[a] Drugs were coadministered.
[b] At least 25 animals were uced to construct each dose-response curve from which morphine ED_{50} values were determined.
* Significantly different from saline- or lithium-treated animals ($P < .05$); † significantly different from morphine + saline-treated animals ($P < .05$).

dose of morphine enhanced tolerance both 6 and 24 hr later as it did at the 3-hr time period (table 2). Administration of 5 mEq/kg of LiCl by itself 3, 6 or 24 hr before test morphine had no effect on the analgesia induced by the test doses of morphine.

Administration of 12 mg/kg of levorphanol also resulted in the development of tolerance to test doses of levorphanol in both the tail-flick and hot-plate assays (table 3). The development of tolerance was detected as a parallel rightward shift in the levorphanol dose-response curve. Administration of 12 mg/kg of levorphanol 3 hr earlier resulted in an increase in the levorphanol ED_{50} of approximately 2.7-fold. Coadministration of 5 mEq/kg of LiCl with the 12-mg/kg dose of levorphanol resulted in a further increase in the levorphanol ED_{50} so that it was 4- to 5-fold greater than the control levorphanol ED_{50} (table 3). As observed previously, lithium treatment by itself 3 hr before test doses of levorphanol had no effect on the levorphanol-induced analgesia in either the tail-flick or the hot-plate assay.

Lithium administered 10 min before test doses of morphine or levorphanol did not significantly affect the ED_{50} of morphine or levorphanol in control, morphine pretreated or levorphanol-pretreated mice (data not shown). Thus, lithium coadministered with morphine or levorphanol affected the development of tolerance to these two opiates and not the expression of tolerance as judged by the induced analgesia.

Effect of apomorphine or lithium on naloxone-precipitated withdrawal. Lithium coadministered in a dose of 5 mEq/kg with 100 mg/kg of morphine or 12 mg/kg of levorphanol 6 or 3 hr, respectively, before naloxone had no significant effect on the ED_{50} of naloxone for inducing a withdrawal jumping response (table 4). However, an effect of lithium was observed when the lithium was used in combination with apomorphine. Apomorphine administered 5 min before naloxone, and thus 5 hr 55 min after 100 mg/kg of morphine or 2 hr 55 min after 12 mg/kg of levorphanol, resulted in a decrease of the naloxone ED_{50} for inducing withdrawal jumping (table 4). Coadministration of 5 mEq/kg of LiCl with 100 mg/kg of morphine 5 hr 55 min before apomorphine increased the naloxone ED_{50} back to control levels. Coadministration of 5 mEq/kg of LiCl with 12 mg/kg of levorphanol 2 hr 55 min before apomorphine also returned the naloxone ED_{50} to control levels. Thus, lithium coadministered with the two opiates attenuated the ability of apomorphine to decrease the ED_{50} of naloxone.

TABLE 3

Effect of lithium on development of tolerance to 12 mg/kg of levorphanol

Treatment[a]	Test Levorphanol ED_{50} (95% CL)[b]	
	Tail flick	Hot plate
	mg/kg s.c.	
3 hr before test levorphanol		
Saline + saline	1.0 (0.9–1.1)	0.9 (0.8–1.1)
5 mEq/kg of LiCl + saline	1.0 (0.9–1.1)	0.9 (0.8–1.1)
Saline + 12 mg/kg of levorphanol	2.8 (2.5–3.1)*	2.4 (2.0–2.9)*
5 mEq/kg of LiCl + 12 mg/kg of levorphanol	4.7 (4.2–5.6)*,†	3.5 (2.9–4.1)*,†

[a] Drugs were coadministered.
[b] At least 24 animals were used to construct each dose-response curve from which levorphanol ED_{50} values were determined.
* Significantly different from saline- or lithium-treated animals (P < .05); † significantly different from levorphanol + saline-treated animals (P < .05).

TABLE 4

Effect of lithium on the potentiation by apomorphine of naloxone-induced withdrawal jumping

Dose of Apomorphine[a]	Naloxone ED_{50} Mean ± S.E. (N)	Naloxone ED_{50} Mean ± S.E. (N)
mg/kg i.p.	*mg/kg i.p.*	*mg/kg i.p.*
	Saline + 100 mg/kg of morphine[b]	5 mEq/kg of LiCl + 100 mg/kg of morphine[b]
0	1.08 ± 0.18 (11)	1.06 ± 0.18 (9)
0.5	0.56 ± 0.12 (9)*,†	1.45 ± 0.26 (8)
	Saline + 12 mg/kg of levorphanol[c]	5 mEq/kg of LiCl + 12 mg/kg of levorphanol[c]
0	0.68 ± 0.09 (9)	0.68 ± 0.09 (9)
0.5	0.38 ± 0.07 (5)*,†	0.69 ± 0.07 (7)

[a] Administered 5 min before naloxone.
[b] Coadministered 6 hr before naloxone.
[c] Coadministered 3 hr before naloxone.
* Significantly different from animals not treated with apomorphine (analysis of variance; means compared by least-significant difference, P < .05); † significantly different from animals which received lithium simultaneously with morphine or levorphanol and the same dose of apomorphine (analysis of variance; means compared by least-significant difference, P < .05).

TABLE 5

Effect of lithium on brain levels of morphine

Dose of Morphine	Time after Treatment	Brain Level of Morphine (ng of Morphine/g of Wet Brain)	
		Saline[a] (N)	5 mEq/kg of LiCl[a] (N)
mg/kg i.p.	*hr*		
10	0.5	241 ± 18 (8)	200 ± 24 (8)
5	0.5	105 ± 9 (8)	88 ± 7 (8)
100 mg/kg of morphine 3 hr before treatment			
30	0.5	952 ± 71 (8)	878 ± 24 (6)
15	0.5	572 ± 42 (8)	597 ± 36 (7)
0	0.5	422 ± 25 (6)	389 ± 30 (7)
0	0	1115 ± 105 (8)	1088 ± 118 (7)

[a] Coadministered with 100 mg/kg of morphine or 3 hr before treatment.

Apomorphine administered in the absence of naloxone did not induce withdrawal jumping.

Lithium administered 10 min before naloxone had no significant effect on the naloxone ED_{50} determined 6 or 3 hr after morphine or levorphanol pretreatment, respectively (data not shown). Lithium administered 5 min before apomorphine (and thus 10 min before naloxone) had no significant effect on the ability of apomorphine to potentiate naloxone-induced jumping. As a result, the naloxone ED_{50} decreased significantly when lithium was administered 10 min before and apomorphine 5 min before naloxone. The resultant naloxone ED_{50} was similar to those shown in table 4 when saline was coadministered with either morphine or levorphanol. Thus, lithium had an effect on the development of dependence to these two opiates and not on the expression of naloxone-induced withdrawal jumping.

Effect of lithium on brain levels of morphine. Administration of 5mEq/kg of LiCl did not have any significant effect on brain levels of morphine after several different treatment protocols (table 5). Lithium administered 3 hr before 10 or 5 mg/kg of morphine did not alter the levels of morphine observed in the brain 30 min later. Coadministration of lithium with 100 mg/kg of morphine did not affect the levels of morphine observed in the brain 3 or 3.5 hr later. Administration of 30 or 15 mg/kg of morphine 3 hr after 100 mg/kg of morphine resulted in brain levels of morphine that were similar in animals that had received saline or lithium concurrently with the 100-mg/

kg dose of morphine. Thus, lithium did not alter the distribution of morphine to the brain.

Effect of acute morphine treatment on [^{3}H]spiroperidol binding. Three hours of treatment of mice with 100 mg/kg of morphine resulted in a parallel shift to the right of the Scatchard plot of [^{3}H]spiroperidol binding. This rightward shift resulted in a significant increase in the maximum binding for [^{3}H]spiroperidol binding sites 3 and 6 hr after morphine administration (table 6). A significant increase in B_{max} also occurred 3 hr after 12 mg/kg of levorphanol. The increase in B_{max}, however, was no longer present 24 hr after the administration of the morphine. Likewise, the increase was not observed at either 3 or 6 hr after 100 mg/kg of morphine when 5 mEq/kg of LiCl was coadministered with the morphine. None of the treatments significantly affected K_d.

Discussion

The results of this study indicate that an acute administration of an opiate to mice can result in the development of supersensitive DA receptors. DA receptor sensitivity was measured both behaviorally using apomorphine-induced climbing behavior, and biochemically using radioligand binding. The behavioral studies indicated that a single administration of morphine or levorphanol could increase the sensitivity of mice to apomorphine whereas the biochemical studies indicated an increase in the number of [^{3}H]spiroperidol binding sites. These results taken together would suggest that acute opiate administration can lead to the development of supersensitive DA receptors.

TABLE 6
Whole brain [^{3}H]spiroperidol binding after 100 mg/kg of morphine or 12 mg/kg of levorphanol
B_{max}, maximum binding.

Treatment	N	B_{max}	K_d
		fmol/mg protein	nM
3 hr			
Saline + saline[a]	8	182 ± 5	0.54 ± 0.03
5 mEq/kg of LiCl + saline[a]	6	180 ± 7	0.62 ± 0.02
Saline + 100 mg/kg of morphine[a]	9	217 ± 7*	0.59 ± 0.05
5 mEq/kg of LiCl + 100 mg/kg of morphine[a]	6	183 ± 9	0.70 ± 0.05
Saline + 12 mg/kg of levorphanol[a]	7	212 ± 6*	0.54 ± 0.05
6 hr			
Saline + saline[a]	6	194 ± 7	0.46 ± 0.04
5 mEq/kg of LiCl + saline[a]	6	186 ± 4	0.58 ± 0.05
Saline + 100 mg/kg of morphine[a]	6	223 ± 4†	0.54 ± 0.03
5 mEq/kg of LiCl + 100 mg/kg of morphine[a]	6	204 ± 4	0.48 ± 0.03
24 hr			
Saline + saline[a]	5	191 ± 4	0.59 ± 0.04
5 mEq/kg of LiCl + saline[a]	6	185 ± 10	0.53 ± 0.03
Saline + 100 mg/kg of morphine[a]	5	180 ± 5	0.55 ± 0.03
5 mEq/kg of LiCl + 100 mg/kg of morphine[a]	6	175 ± 5	0.63 ± 0.04

[a] Coadministered at the time indicated.
* Significantly different from the other values in the group except for not being significantly different from the other saline + opiate-treated group (analysis of variance; means compared by least-significant difference, $P < .05$); † significantly different from the other values in the same group (analysis of variance; means compared by least-significant difference, $P < .05$).

It could be argued that the [^{3}H]spiroperidol binding sites represented an alteration in the number of serotonin receptors and not in the number of DA receptors. However, several previous findings would not be consistent with an alteration in serotonin. First, our previous findings showed an increase in [^{3}H]spiroperidol binding sites only in the brain region which included the ventral and dorsal striatum; no alterations in binding sites were found in several other brain regions including the frontal cortex (Martin and Takemori, 1985). Spiroperidol has been shown to bind serotonin receptors of the frontal cortex, but not those of the striatum (McGonigle *et al.*, 1984). Second, serotonin agonists have an inhibitory effect on apomorphine-induced climbing behavior in mice (Costall *et al.*, 1978; Wilcox *et al.*, 1978). An increase in serotonin receptors might then be expected to result in an inhibition of apomorphine-induced climbing behavior rather than the observed increase. Therefore, the increase in [^{3}H]spiroperidol binding sites was most probably due to an increase in DA receptors.

An increase in [^{3}H]spiroperidol binding sites was observed 3 and 6 hr after administration of 100 mg/kg of morphine and 3 hr after administration of 12 mg/kg of levorphanol. However, an enhancement of apomorphine-induced climbing behavior was not observed 3 hr after the 100 mg/kg of morphine. The reason for this lack of enhancement is unclear at this time, but may have been due to a masking of the apomorphine effect by the locomotor stimulatory action of morphine which was still present at this time. This morphine-induced locomotor stimulation is present up to 5 hr after 100 mg/kg of morphine (P. C. Contreras and A. E. Takemori, unpublished results). A lower dose of morphine (10 mg/kg), however, can enhance apomorphine-induced climbing behavior and increase the density of [^{3}H]spiroperidol binding sites 3 hr after morphine administration (Martin and Takemori, 1985). In contrast, an enhancement of apomorphine-induced climbing behavior was still present 24 hr after morphine administration when [^{3}H]spiroperidol binding sites were observed to have returned to control levels. The reason for this is not entirely clear.

Evidence obtained in recent studies have indicated that alterations in DA receptor numbers and/or affinity may not be that closely related to DA receptor sensitivity. Instead DA receptor sensitivity may be related more closely to the amount of DA available to the receptor. The availability of DA to its receptor has been suggested to influence the adaptational state and/or postreceptor functions associated with the DA receptor (Carlsson, 1983; Clark *et al.*, 1985a,b). Furthermore, evidence was presented which suggests that these changes can be accompanied by alterations in DA receptor number and/or affinity. This is in contrast to other studies which indicate a relationship exists between DA receptor density and sensitivity (Fleminger *et al.*, 1983). In the present study, there was: 1) an increase in the number of spiroperidol binding sites without an accompanying change in sensitivity to apomorphine 3 hr after morphine and 2) a return to control levels of binding site density in the presence of enhanced sensitivity to apomorphine 24 hr after morphine. These results may reflect the lack of correlation between DA receptor sensitivity and density and/or affinity of DA receptors. Such a lack of correlation may explain why some previous studies have indicated the development of supersen-

sitive DA receptors after chronic morphine with no changes in radioligand binding (Christie and Overstreet, 1979). Thus, it may be possible that supersensitive DA receptors can develop after opiate treatment without changes in either receptor density or affinity. The exact mechanisms by which opiates induce DA receptor supersensitivity may have to await full understanding of those mechanisms responsible for altering DA receptor sensitivity.

Further evidence that acute morphine or levorphanol administration can induce the development of supersensitive DA receptors were obtained from the results of lithium coadministration. Coadministration of lithium with morphine or levorphanol attenuated both the enhancement of apomorphine-induced climbing behavior and the increase in the number of [^{3}H]spiroperidol binding sites. These results are similar to those obtained during chronic neuroleptic treatment. Haloperidol treatment over a 2-week period results in an increase in sensitivity to apomorphine and an increase in [^{3}H]spiroperidol binding sites. Concurrent lithium treatment with haloperidol has been shown to attenuate both the increased sensitivity to apomorphine and the increase in [^{3}H]spiroperidol binding sites (Pert *et al.*, 1978; Creese and Snyder, 1980). The difference in the time course of the action of lithium on haloperidol-induced (2 weeks) and opiate-induced (several hours) DA receptor supersensitivity might be due to differences in the time course of action of haloperidol and that of an opiate. Haloperidol requires approximately 2 weeks in which to exert its therapeutic effect. The therapeutic action of haloperidol has been theorized recently to be due to depolarization inactivation of the dopaminergic neurons which arise in the ventral tegmental area and project to the nucleus accumbens (White and Wang, 1983; Mereu *et al.*, 1985). It takes approximately 2 weeks for haloperidol-induced depolarization inactivation to develop (White and Wange, 1983; Mereu *et al.*, 1985). At the time of depolarization inactivation, DA release would be decreased, and the numbers of DA receptors would be expected to have increased resulting in DA receptor supersensitivity. Before depolarization inactivation, DA neuron activity would be enhanced and DA release would be increased which, in turn, would counteract the early action of haloperidol.

Opiates may have a similar effect on DA neuronal activity and DA release except on a much shorter time course. Morphine has been observed by some groups to enhance DA release (Chesselet *et al.*, 1981a,b, 1982, 1983; Wood *et al.*, 1980) and by others to inhibit DA release (Loh *et al.*, 1976; Sparber *et al.*, 1979). Electrophysiological studies have indicated that dopaminergic neuronal activity increases after morphine administration (Gysling and Wang, 1983; Matthews and German, 1984). This would be expected to result in an increase in DA release. However, further study on the effect of morphine on DA neuron activity indicates the initial increase in activity is followed by what appears to be depolarization inactivation (Hu and Wang, 1984). This would be expected to result in a decrease in DA release. *In vivo* voltametry over a 3-hr time period showed an initial increase in release of striatal DA at 1 hr followed by a decrease in release at 2 and 3 hr (Broderick, 1985). Thus, opiates might cause an immediate increase in DA neuron activity accompanied by an increase in DA release. This might then be followed by depolarization blockade and an accompanying decrease in DA release. The decrease in DA release after the initial increase might be responsible for the opiate-induced development of supersensitive DA receptors. This hypothesis requires further study.

The results of the present study also indicate that opiate-induced DA receptor sensitivity might affect the degree of analgesic tolerance which develops to the opiate. Coadministration of lithium with either morphine or levorphanol resulted in a greater degree of analgesic tolerance which corresponded most closely with the attenuation of increased sensitivity to apomorphine. However, lithium might have enhanced tolerance by several other mechanisms. First, lithium might have decreased the distribution of morphine to the brain so that a greater dose of morphine would have been necessary in order to obtain the same brain level of morphine and the same degree of analgesia. However, several different administration schedules of lithium and morphine, all of which were identical to the administration schedules used to test analgesic tolerance, failed to significantly alter morphine brain levels. Second, lithium might have affected the binding and interaction of morphine with its receptor. However, binding studies done on rats have shown that acute lithium administration does not affect opiate binding parameters (Wajda *et al.*, 1981). Furthermore, lithium administered alone 3 hr before morphine did not alter morphine-induced analgesia indicating that lithium did not interfere with the interaction of morphine with its receptor. Therefore, it seems unlikely that lithium affected the development of analgesic tolerance by affecting the interaction of the opiate with its receptor, or by altering brain levels of morphine.

Our observation that the blockade of the development of supersensitive DA receptors by lithium enhances analgesic tolerance is in disagreement with previous reports that suggest that supersensitive DA receptors might decrease the degree of opiate-induced analgesic tolerance. It has been reported that the administration of PLG (Bhargava, 1980) or cyclo(leucylglycine) (Ritzmann *et al.*, 1979, 1982a,b; Bhargava, 1980) prevents the development of morphine-induced DA receptor supersensitivity while simultaneously decreasing the degree of analgesic tolerance. It has since been shown that intracerebroventricular injections of low concentrations of PLG can facilitate whereas higher concentrations can inhibit the development of analgesic tolerance (Contreras and Takemori, 1981, 1984) The facilitation of tolerance by PLG is similar to that caused by lithium. It is interesting that PLG has been reported to attenuate the development of supersensitive DA receptors caused by chronic neuroleptic treatment (Chiu *et al.*, 1981). Thus, the facilitation of analgesic tolerance by PLG may be due to the blockade of the development of supersensitive DA receptors induced by opiate administration. PLG has also been shown to have weak affinity for the opiate receptor (Contreras and Takemori, 1984) and this effect may be responsible for the blockade of DA receptor supersensitivity, and the inhibition of analgesic tolerance observed in the earlier studies.

Further evidence for the development of supersensitive DA receptors was obtained from the results of naloxone-induced withdrawal jumping. This jumping syndrome has been linked to the development of dependence (Maggiolo and Huidobro, 1961), and has become a valuable tool in the assessment of physical dependence upon opiates (Way *et al.*, 1969). Furthermore, several reports implicate the involvement of DA in this syndrome (Iwamoto *et al.*, 1973; Maruyama and Takemori, 1973). In the present study apomorphine admnistration decreased the amount of naloxone required to induce withdrawal jumping in mice previously administered morphine or

levorphanol. Lithium administered concurrently with the opiate blocked the enhancing effect of apomorphine on naloxone. These results are interpreted as an indication of the involvement of DA and supersensitive DA receptors in the naloxone-induced withdrawal syndrome. This suggestion will require further rigorous testing before the exact role of DA in physical dependence and withdrawal is known.

In summary, the results of the present study indicate the development of supersensitive DA receptors after the administration of an opiate to mice. This DA receptor supersensitivity modifies the degree of analgesic tolerance and physical dependence which develops in response to the opiate administration.

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