Pharmacology Biochemistry & Behavior, Vol. 16, pp. 119–124, 1982. Printed in the U.S.A.

Interaction of Opiates with Dopamine Receptors: Receptor Binding and Behavioral Assays

KRISTIN R. CARLSON AND THOMAS F. SEEGER[1]

Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605

Received 6 April 1981

CARLSON, K. R. AND T. F. SEEGER. *Interaction of opiates with dopamine receptors: Receptor binding and behavioral assays.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 119–124, 1982.—We examined the hypothesis that opiates act as dopamine (DA) receptor-blocking agents thereby inducing a compensatory increase in DA receptor density during chronic administration, and that increased receptor density could account for the behavioral hypersensitivity to DA agonists seen after treatment with opiates. Morphine and methadone did not inhibit the specific binding of ^{3}H-spiroperidol to DA receptors *in vitro*, nor did they decrease affinity or apparent receptor density in the striatum when administered acutely *in vivo* in behaviorally effective doses. In contrast, neuroleptics had the expected inhibitory effect in both these experiments. Stereotypy and locomotion in response to apomorphine were measured before and after a 3-week treatment with saline or methadone. About half the methadone-treated rats showed significant increases over predrug baselines in stereotypy or locomotion, as did a few saline-treated animals. However, in those animals showing enhanced stereotypy or locomotion, DA receptor density was not elevated in striatum or mesolimbic areas respectively. These results indicate that opiates do not act as antagonists at DA receptor sites, and that changes in DA receptor density cannot account for opiate-induced behavioral hypersensitivity.

Opiates Dopamine Stereotypy Locomotion Methadone ^{3}H-spiroperidol Striatum Mesolimbic area Hypersensitivity

CHRONIC treatment with opiates can induce hypersensitivity of the dopaminergic nigrostriatal system, as expressed in enhanced stereotypic gnawing and chewing in response to dopaminergic agonists following termination of this treatment [6, 7, 8, 17, 18]. This hypersensitivity is very similar behaviorally to the syndrome produced by chronic neuroleptic treatment in animals [41,48] and in humans, where it is thought to be the underlying cause of tardive dyskinesia [2,19]. Neuroleptic-induced hypersensitivity is thought to reflect a proliferation of post-synaptic dopamine (DA) receptors secondary to their chronic blockade by neuroleptics. All clinically effective neuroleptics act as antagonists at DA recptors [11,46], and, in animals, receptor density in striatum is increased by neuroleptic administration [4,30]. The extent of the increase is positively correlated with drug dosage [10] and duration of treatment [16,32]. In addition to their ability to induce enhanced stereotypy, opiates and neuroleptics share other actions suggestive of DA receptor blockade such as induction of catalepsy [1, 24, 40], increased DA turnover [1, 24, 34, 40], and antagonism of the behavioral effects of amphetamine [17, 38, 44]. These similarities have led several researchers to propose that both groups of drugs act directly as antagonists at post-synaptic DA receptors [6, 37, 38].

Several tests of this hypothesis are possible using the binding of ^{3}H-neuroleptics to DA receptors in homogenates of striatum or other DA-rich areas as an assay system. First, one can add opiates to the homogenate and assess the extent to which they occupy receptors *in vitro*, thereby displacing the ^{3}H-neuroleptic. Recent reports [12, 26, 33] indicate that opiates are ineffective in this regard; the present study replicates these results. Second, one can administer opiates in physiological doses acutely *in vivo*, and at the time of peak behavioral effect, when brain concentrations are presumably maximal, sacrifice the animal and measure any displacement of binding of ^{3}H-neuroleptics. In this study we investigated the effects of morphine, methadone, and dopaminergic drugs as controls.

Finally, the role of changes in DA receptor density or affinity in the phenomenon of behavioral hypersensitivity after chronic treatment can be assessed. The existing studies bearing on this topic are contradictory. Morphine pellet implantation for 3–5 days produced a slight increase in DA receptor density in striatum 8 hr after pellet removal [14], but a decrease 24 and 48 hr after removal [39]. A 104-day course of morphine injections, on the other hand, caused no change in receptor density 23 hr after withdrawal [9]. An additional factor which complicates the interpretation of these results is that no measure of behavioral hypersensitivity to DA agonist challenge was made in one study [39], and was made in subjects other than those used for the binding assay in the other two studies [9,14]. Although this is rarely done, both recep-

[1]Present address: Department of Psychiatry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

Copyright © 1982 ANKHO International Inc.—0091-3057/82/010119-06$03.00/0

tor changes and behavioral alterations should be demonstrated in the same animal [31]. The value of a subject-by-subject analysis is that receptor density changes can be evaluated with respect to the presence or absence of hypersensitivity, which may be induced in some animals but not in others by the same chronic drug regimen. In other words, the correlation is between receptor density and exhibition of a target behavior, rather than between receptor density and having undergone drug treatment. If opiates block DA receptors, the extent to which DA receptor density is elevated over control levels would be positively correlated with the degree of behavioral hypersensitivity.

We have examined the relationship of behavior to receptor changes in two dopaminergic systems, the nigrostriatal, primarily responsible for stereotyped behavior [22], and the mesolimbic, primarily responsible for locomotor activity [23]. As discussed earlier, both neuroleptics and opiates can induce behavioral hypersensitivity in the nigrostriatal system; in the mesolimbic system, neuroleptics also induce hypersensitivity [13,45], but the effects of opiates have not been investigated. We measured stereotypy and locomotion in response to apomorphine challenge before and after chronic saline or opiate treatment, and then assayed DA receptor density in striatum and mesolimbic areas in each of these subjects. ^{3}H-spiroperidol was chosen as the ligand, since it and the other butyrophenone ^{3}H-haloperidol have been the ligands of choice in assessing post-synaptic receptor changes due to chronic neuroleptic [4, 10, 16, 30, 32] or opiate [9,39] treatment. The opiate selected was methadone, since it is used extensively on a chronic basis in methadone maintenance treatment programs.

METHOD

Subjects

Male albino Holtzman rats (Charles River) were used. They weighed 320–350 g at the time of sacrifice for the *in vitro* and acute *in vivo* studies, and at the beginning of the chronic drug study. They were housed individually with free access to Purina chow and tap water. An automatic timer maintained a 7 a.m. on/7 p.m. off light cycle. During the chronic drug study Ss were weighed every other day.

Drugs

The following drugs were used: apomorphine HCl (Sigma), (+) and (−)butaclamol (courtesy of Ayerst), haloperidol (courtesy of McNeil), methadone HCl powder and liquid for injection (Lilly), morphine sulfate (Lilly), and naloxone HCl (courtesy of Endo). Powdered drugs were diluted in glass-distilled water to the proper concentrations as the salt, with pH adjustment as necessary.

In Vitro *Inhibition of Specific Binding*

Rats were decapitated, the brains rapidly removed onto a glass plate over ice and the striata separated by blunt dissection. The tissue was weighed and homogenized in 100 vol of ice-cold buffer (0.5 M monobasic and dibasic potassium phosphate in 0.9% saline, pH=6.9 at 4°C) with a Brinkman Polytron (setting 7, two 5-sec bursts). The suspension was centrifuged at 15,000 rpm for 10 min, and the pellet rehomogenized in fresh buffer. Centrifugation and rehomogenization in 100 vol fresh buffer was repeated. Incubation tubes in duplicate were prepared with 0.8 ml tissue suspension, 0.1 ml of 10 nM ^{3}H-spiroperidol (23.6 Ci/mmole, New England Nuclear), and 0.1 ml of an appropriate concentration of an inhibiting drug. Specific binding was defined as the difference between tubes containing no inhibiting drug and tubes containing 0.1 ml of 10 μM (+)butaclamol. Tubes were incubated with agitation in a 37°C water bath for 10 min. Reactions were terminated by rapid vacuum filtration onto GF/B filters, followed by a 5 ml ice-cold saline rinse, a 2 ml tube rinse, and a final 5 ml rinse of the filter. The filters were extracted in 4 ml Aquasol in minivials and counted at 38–40% efficiency.

Once the appropriate range of concentration had been determined (the highest final concentration used was 1×10^{-2} M), triplicate assays were performed for each inhibiting drug, using at least four concentrations/assay, and the results averaged. These data, expressed as percentage inhibition of specific binding, were converted to probits and IC_{50}s were determined by linear regression analysis.

In Vivo *Inhibition of Specific Binding*

Rats were injected SC with either saline (1.0 ml/kg), haloperidol (1.0 mg/kg), morphine (10 mg/kg), or methadone (10 mg/kg). At the time of peak behavioral effect, 30 min post-injection, Ss were sacrificed. In addition, a group receiving 1.0 mg/kg apomorphine was sacrificed 24 hr post-injection to determine whether this delay would be sufficient to prevent any interference with the binding assay by residual apomorphine, since in the chronic drug study we planned to administer this dose of apomorphine 24 hr before sacrifice. Striata were removed and tissue from two animals was pooled for assay. Tissue homogenates were prepared as above. Incubation tubes in duplicate contained 0.8 ml tissue homogenate, 0.1 ml ^{3}H-spiroperidol (final concentrations 0.25–5.0 nM), and 0.1 ml water or (+)butaclamol in a 1000:1 ratio to the corresponding ^{3}H-spiroperidol concentration. Incubations, filtrations, and liquid scintillation counting were performed as above. A Lowry protein assay [28] was performed on every homogenate. Three separate assays were done for each drug and the results averaged. Affinity (K_D) and receptor density (B_{max}) were determined by Scatchard analysis [3], and differences were tested by one-way analyses of variance with subsequent Newman-Keuls test [49].

Chronic Drug Effects on Behavior and Specific Binding

The behavioral effects of apomorphine were assessed in an identical fashion on the day before chronic drug treatment was begun and on the 14th day following the termination of treatment. During the lights-on hrs each S was injected IP with 0.5 mg/kg apomorphine and placed in a 60×60×30 cm high Plexiglas box with a mesh floor. They were observed for 3 2-min periods beginning at 5, 15, and 25 min post-injection, during which the durations of stereotyped sniffing and chewing, and the control behaviors grooming and rearing, were separately recorded by a keyboard device with associated timers. Only the chewing data are presented, since almost all Ss exhibited continuous sniffing during both test sessions, and there were no consistent changes in grooming and rearing. In addition, the number of crossing, i.e., the number of times S moved with four feet from one quadrant to another on the floor of the test box, were recorded. Chewing durations were averaged across the three test periods, since there were no trends over time.

During the chronic drug phase, Ss were injected SC for three weeks with sterile 0.9% saline (SAL; 1.0 ml/kg) or

sterile methadone HCl for injection (MD; 10 mg/kg/day during week 1, 15 mg/kg/day during week 2, and 20 mg/kg/day during week 3). The SAL group received 1 injection/day, and the MD group received half the daily dose in each of two injections separated by 12±2 hr. Initial Ns were SAL=10 and MD=14; all Ss completed the study.

On the day following the post-treatment apomorphine challenge, Ss were decapitated and the brain rapidly removed onto a glass plate over ice. With the brain resting on its dorsal surface, parallel vertical knife cuts were made anterior and posterior to the olfactory tubercle, and the resulting slice between the cuts was dissected bilaterally into striata and mesolimbic area (nucleus accumbens and olfactory tubercle). Samples were immediately frozen on dry ice and stored at −70°C until assayed.

Since the rationale of this experiment was to correlate behavioral change with DA receptor density, each group was subdivided into those Ss which increased in duration of chewing and those Ss which decreased or showed no change, as compared to their pre-chronic drug behavior. Each group was similarly subdivided on the basis of increases in number of crossings.

Because each S was being assayed separately, the amount of tissue was only sufficient for a determination of DA receptor density using one ^{3}H-spiroperidol concentration. A sample from each subgroup was run in each assay. Tissue homogenates were prepared as usual, and incubations were performed in triplicate. The final ^{3}H-spiroperidol concentration for striatal samples was 1.0 nM and for mesolimbic samples 2.0 nM. Other technical aspects of the binding assay were as described for the acute *in vivo* assay. A Lowry protein assay was performed on each tissue homogenate, and data were expressed as fmoles specifically bound/mg protein. Two SAL mesolimbic samples and one MD striatal sample gave aberrant results and were excluded as outliers from data analysis [15]. Receptor density and behavioral differences among the subgroups were tested by one-way analyses of variance [49].

TABLE 1

FINAL CONCENTRATIONS INHIBITING SPECIFIC BINDING BY 50% (IC_{50}) *IN VITRO*

Drug	IC_{50} (M)
(+)Butaclamol	1.28×10^{-8}
Haloperidol	2.32×10^{-8}
Apomorphine	3.00×10^{-6}
(−)Butaclamol	5.06×10^{-5}
Methadone	3.17×10^{-4}
Morphine	$>1.0\times10^{-2}$
Naloxone	$>1.0\times10^{-2}$

TABLE 2

AFFINITY (K_D) AND DENSITY (B_{MAX}) OF DOPAMINE RECEPTORS IN STRIATUM AFTER ACUTE *IN VIVO* DRUGS

Drug	K_D (nM)*	B_{max} (fm/mg protein)†
Saline	0.09 ± 0.01	626.10 ± 14.04
Haloperidol	0.72 ± 0.05‡	559.56 ± 42.12
Morphine	0.10 ± 0.01	603.60 ± 88.39
Methadone	0.08 ± 0.02	685.70 ± 58.56
Apomorphine (after 24 hrs)	0.09 ± 0.00	633.37 ± 47.30

Values are Mean ± SEM.
*$F(4,9)=133.0$, $p<0.001$.
†$F(4,9)<1.0$, ns.
‡$p<0.01$ compared to the Saline group by Newman-Keuls test.

RESULTS

In Vitro *Inhibition of Specific Binding*

The concentrations which inhibited specific binding by 50% (IC_{50}) are shown in Table 1. The neuroleptics (+)butaclamol and haloperidol were very potent, and the agonist apomorphine somewhat less so, as expected. There was a detectable inhibition by methadone, but it cannot be considered of physiological significance, since it occurred at a higher concentration than that required by the inactive isomer (−)butaclamol. Neither morphine nor naloxone produced any detectable inhibition at concentrations up to 1.0×10^{-2} M, indicating that these substances do not react with DA receptor sites.

In Vivo *Inhibition of Specific Binding*

At the time of sacrifice, Ss administered haloperidol, morphine, and methadone were appropriately cataleptic. Saline Ss showed no behavioral abnormalities, nor did the apomorphine Ss upon sacrifice 24 hr post-injection.

As shown in Table 2, Scatchard analysis of the data indicated that acute treatment with haloperidol significantly reduced receptor affinity, i.e., increased K_D, indicating competitive inhibition of binding. The haloperidol-induced decrease in affinity was primarily the result of residual haloperidol in the tissue homogenate, since preparing the tissue with an additional two washes by centrifugation raised affinity to near-control levels ($K_D=0.20\pm0.005$). However, this affinity was still lower than that of identically-prepared tissue derived from saline-injected animals ($K_D=0.12\pm0.01$; $t(2)=11.21$, $p<0.01$). The additional centrifugations had no effect on B_{max} in either group (data not shown).

Apomorphine 24 hr before sacrifice did not affect specific binding; thus, its use in the chronic drug experiment on the day prior to sacrifice is justified.

The major result of this experiment is that the presence of morphine or methadone in the striatal homogenates had no effect on DA receptor density or affinity. This finding is consistent with the *in vitro* data, and indicates that these opiates do not act as antagonists at DA receptor sites in the striatum.

Chronic Drug Effects on Behavior and Specific Binding

Over the course of chronic drug administration, the saline-injected Ss gained weight steadily, whereas the methadone-injected Ss lost an average of 20 g the first week and had not regained their initial weight by the end of drug administration. The latter Ss exhibited a further average 10 g decrease during the first two withdrawal days, indicating that they were physically dependent, but began gaining weight thereafter. On the post-treatment apomorphine challenge day the average body weights were SAL=475 g, MD=395 g.

TABLE 3

STEREOTYPED CHEWING AND DOPAMINE RECEPTOR DENSITY IN STRIATUM AFTER CHRONIC SALINE OR METHADONE

Group	Change from Pre-Chronic Drug	N	Chewing Duration* (sec)	Receptor Density† (fm/mg protein)
SAL	Increased	3	14.37 ± 4.69	169.71 ± 53.93
	Decreased	7	1.46 ± 0.47	200.58 ± 20.60
MD	Increased	7	13.65 ± 2.39	194.05 ± 34.43
	Decreased	6	5.69 ± 0.93	232.71 ± 16.99

Values are Mean ± SEM.
*$F(3,19)=10.49$, $p<0.001$.
†$F(3,19)<1.0$, ns.

TABLE 4

LOCOMOTION AND DOPAMINE RECEPTOR DENSITY IN MESOLIMBIC AREA AFTER CHRONIC SALINE OR METHADONE

Group	Change from Pre-Chronic Drug	N	Crossings* (No.)	Receptor Density† (fm/mg protein)
SAL	Decreased	8	4.38 ± 1.29	194.91 ± 18.02
MD	Increased	7	15.57 ± 3.14	201.42 ± 15.02
	Decreased	7	7.86 ± 1.32	209.19 ± 8.05

Values are Mean ± SEM.
*$F(2,19)=7.90$, $p<0.005$.
†$F(2,19)<1.0$, ns.

The SAL and MD groups did not differ in duration of chewing at the post-chronic drug apomorphine challenge, $t(21)=1.63$, ns. However, as shown in Table 3, more MD than SAL Ss increased in duration of chewing, and there was a significant overall difference among the subgroups in duration of chewing post-chronic drug (there was no difference pre-chronic drug: $F(3,19)=2.17$, ns). However, there was no difference among the subgroups in density of striatal DA receptors, indicating that significant behavioral difference was not accompanied by a change in receptor density.

Regarding locomotion, the SAL and MD groups were significantly different in number of crossings post-chronic drug, $t(20)=2.64$, $p<0.02$, primarily because all the SAL Ss decreased whereas half the MD Ss increased in number of crossings compared to pre-chronic drug levels. The subgroups as well were significantly different in number of crossings post-chronic drug, as shown in Table 4, although they had not differed before chronic drug treatment, $F(2,19)<1.0$, ns. This behavioral difference was not accompanied by any alteration in density of mesolimbic DA receptors, however.

Further, increases or decreases in chewing or locomotion, independent of chronic drug treatment, were not related to receptor density in the relevant areas. Comparison of both SAL- and MD-treated Ss which increased with Ss from both groups which decreased showed no receptor density differences in striatum, $t(21)=0.99$, ns, or mesolimbic area, $t(20)=0.01$, ns, indicating that behavioral change per se is not correlated with receptor density.

DISCUSSION

In the *in vitro* and acute *in vivo* experiments, as expected, neuroleptics displaced ^{3}H-spiroperidol from its binding sites. Opiates, however, did not compete for DA receptor sites, or did so with such low affinity that the interaction did not affect binding. Thus, it is highly unlikely that morphine and methadone act as antagonists at post-synaptic DA receptors when present in the brain at physiological levels.

These results complement other recent findings. Various behaviors induced by the direct post-synaptic agonist apomorpine are not blocked by doses of morphine which are effective against the same behaviors when elicited by the presynaptic agonist d-amphetamine; these include rotation in the rat lesioned unilaterally in the substantia nigra with 6-hydroxydopamine [47] and stereotypy [44]. Further, morphine is ineffective in disrupting a DA-mediated stimulus control task which is suppressed by chlorpromazine [42].

The more likely site of action of opiates within dopaminergic systems is presynaptic. The demonstration of enkephalin receptors on the axon terminals of DA neurons in striatum [36,43] and mesolimbic areas [35] confirms the anatomical possibility of this type of interaction. The precise nature of the effect exerted via this mechanism remains controversial, some authors proposing that opiates facilitate the release of DA [25,29] but the majority claiming that release is inhibited [5, 27, 36, 43, 47]. The latter role of opiates in the regulation of DA release would be consistent with their ability to produce behavioral hypersensitivity, in that a func-

tional deficiency of DA post-synaptically would have the same net result as receptor blockade. Consequently, opiates might induce post-synaptic receptor proliferation even with a presynaptic site of action.

We found, however, no evidence to support an increase in DA receptor density as the basis for behavioral hypersensitivity following chronic methadone treatment. It might be argued that the duration of treatment was too short or the post-treatment interval too long to produce a detectable effect. These are parameters which could be investigated, since they have been shown to affect binding results following chronic neuroleptic treatment [10, 16, 32].

Another potential explanation of our failure to find changes in receptor density might be a deficiency in specificity or sensitivity of the binding assay itself. However, the *in vitro* data validate the pharmacological specificity of the assay, and the acute *in vivo* data were obtained from assays which confirmed that binding was of high affinity, specific, and saturable. Since ^{3}H-spiroperidol binds to both DA and serotonin receptors [21], it is conceivable that DA receptor changes were obscured by a lack of alterations in serotonin receptors. However, in preparatory work for this experiment we found that a 3-week treatment with haloperidol (1.0 mg/kg IP) produced a significant 26% elevation in striatal ^{3}H-spiroperidol binding sites compared to saline control rats, a value completely in line with other reports [4,30]. Thus, we are confident that if differences existed in the chronic drug experiment they would have been revealed by the assay. Nonetheless, it would be worthwhile to repeat this experiment using ^{3}H-apomorphine as the ligand, since increases in its binding sites in both striatum and mesolimbic areas are of greater magnitude than increases in ^{3}H-haloperidol sites following chronic haloperidol treatment [30].

Under these chronic treatment conditions, methadone produced hypersensitivity of both dopaminergic systems, as expressed behaviorally. The induction by opiates of hypersensitivity in the nigrostriatal system is well-documented [6, 7, 8, 17, 18]; to our knowledge, however, this is the first report of the same phenomenon in the mesolimbic system. It might be argued that the phenomenon was not robust, since it did not occur in all MD-treated Ss. In our experience, this is to be expected under the present dosing regimen. More importantly, we were thereby able to distinguish those Ss which had been rendered behaviorally hypersensitive from those which had not, and, for the first time, evaluate receptor densities on a subject-by-subject basis as a function of the presence of behavioral hypersensitivity, rather than as a function solely of drug treatment. The results with regard to both the nigrostriatal and mesolimbic systems were clear: enhanced responses to apomorphine were not accompanied by elevated DA receptor densities.

If proliferation of post-synaptic DA receptors is not responsible for behavioral hypersensitivity, as our results lead us to believe, there are alternative hypotheses. It is always possible that an increase in DA receptor affinity is responsible. Because our experimental design required that we assay each S individually we were not able to examine this possibility, since each S did not yield enough tissue for a saturation assay. However, a pervasive theme in the general field of drug-induced changes in receptor status is that when alterations are observed they are usually in B_{max} rather than K_D [20].

More likely, in our opinion, are alterations in presynaptic dopaminergic functions. For example, reserpine, which depletes presynaptic stores of DA, produces behavioral hypersensitivity [48]. A logical proposition is that opiates interact with the enkephalin receptors which quite likely play an inhibitory role in the regulation of DA release [5, 27, 35, 36, 43, 47]. If these receptors become subsensitive as a consequence of chronic stimulation by opiates, theoretically this could produce less inhibition of release and consequently a supranormal tonic release level of DA following treatment. By itself the excess DA might not produce any obvious behavioral effect, but would be additive with the direct postsynaptic agonist action of apomorphine, giving rise to behavioral manifestations of hypersensitivity upon challenge.

ACKNOWLEDGEMENTS

Supported by USPHS grant DA02226 and by a grant from the Scottish Rite Schizophrenia Research Program. The technical assistance of John Almasi, Lorraine Martinez, James McCabe, and Yvonne Goldsberry is gratefully acknowledged. We thank Dr. Douglas Waud for a critical review of the manuscript.

REFERENCES

1. Ahtee, L. Catalepsy and stereotypies in rats treated with methadone: Relation to striatal dopamine. *Eur. J. Pharmac.* **27:** 221–230, 1974.
2. Baldessarini, R. J. and D. Tarsy. Dopamine and the pathophysiology of dyskinesias induced by antipsychotic drugs. *A. Rev. Neurosci.* **3:** 23–41, 1980.
3. Bennett, J. P., Jr. Methods in binding studies. In: *Neurotransmitter Receptor Binding*, edited by H. I. Yamamura, S. J. Enna and M. J. Kuhar. New York: Raven Press, 1978, pp. 57–90.
4. Burt, D. R., I. Creese and S. H. Snyder. Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. *Science* **196:** 326–328, 1977.
5. Carenzi, A., A. Guidotti, A. Revuelta and E. Costa. Molecular mechanisms in the action of morphine and viminol (R_2) on rat striatum. *J. Pharmac. exp. Ther.* **194:** 311–318, 1975.
6. Carlson, K. R. Narcotic mechanisms and dyskinesias. In: *Animal Models in Psychiatry and Neurology*, edited by I. Hanin and E. Usdin. New York: Pergamon Press, 1977, pp. 391–403.
7. Carlson, K. R. and J. Almasi. Behavioral supersensitivity to apomorphine following chronic narcotic treatment in the guinea pig. *Psychopharmacology* **57:**273–277, 1978.
8. Carlson, K. R. and J. Almasi. Time course of dopaminergic hypersensitivity following chronic narcotic treatment. *Pharmac. Biochem. Behav.* **11:** 283–287, 1979.
9. Christie, M. J. and D. H. Overstreet. Sensitivity of morphine-tolerant rats to muscarinic and dopaminergic agonists: Relation to tolerance or withdrawal. *Psychopharmacology* **65:** 27–34, 1979.
10. Clow, A., A. Theodorou, P. Jenner and C. D. Marsden. Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. *Eur. J. Pharmac.* **63:** 145–157, 1980.
11. Creese, I., D. R. Burt and S. H. Snyder. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* **192:** 481–483, 1976.
12. Czlonkowski, A., V. Hollt and A. Herz. Binding of opiates and endogenous opioid peptides to neuroleptic receptor sites in the corpus striatum. *Life Sci.* **22:** 953–962, 1978.
13. Davis, K. L., L. E. Hollister and W. C. Fritz. Induction of dopaminergic mesolimbic receptor supersensitivity by haloperidol. *Life Sci.* **23:** 1543–1548, 1978.

14. de la Baume, S., G. Patey, H. Marcais, P. Protais, J. Costentin and J. C. Schwartz. Changes in dopamine receptors in mouse striatum following morphine treatment. *Life Sci.* **24:** 2333–2342, 1979.
15. Dixon, W. J. and F. J. Massey, Jr. *Introduction to Statistical Analysis.* New York: McGraw-Hill, 1969, pp. 328–330.
16. Ebstein, R. P., D. Pickholz and R. H. Belmaker. Dopamine receptor changes after long-term haloperidol treatment in rats. *J. Pharm. Pharmac.* **31:** 558–559, 1979.
17. Eibergen, R. D. and K. R. Carlson. Behavioral evidence for dopaminergic supersensitivity following chronic treatment with methadone or chlorpromazine in the guinea pig. *Psychopharmacology* **48:** 139–146, 1976.
18. Eibergen, R. D. and K. R. Carlson. Dyskinesias in monkeys: Interaction of methamphetamine with prior methadone treatment. *Pharmac. Biochem. Behav.* **5:** 175–187, 1976.
19. Gerlach, J. Tardive dyskinesia. *Dan. med. Bull.* **26:** 209–245, 1979.
20. Gnegy, M. E. and E. Costa. Catecholamine receptor supersensitivity and subsensitivity in the central nervous system. In: *Essays in Neurochemistry and Neuropharmacology*, vol. 4, edited by M. B. H. Youdim, W. Lovenberg, D. F. Sharman and J. R. Lagnado. New York: John Wiley, 1980, pp. 249–282.
21. Iverson, L. L., M. Quik, P. C. Emson, J. E. Dowling and K. J. Watling. Further evidence for the existence of multiple receptors for dopamine in the central nervous system. In: *Receptors for Neurotransmitters and Peptide Hormones*, edited by G. Pepeu, M. J. Kuhar and S. J. Enna. New York: Raven Press, 1980, pp. 193–202.
22. Kelly, R. H., P. W. Seviour and S. D. Iverson. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.* **94:** 507–522, 1975.
23. Koob, G. F., S. J. Riley, S. C. Smith and T. W. Robbins. Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity and amphetamine anorexia in the rat. *J. comp. physiol. Psychol.* **92:** 917–927, 1978.
24. Kuschinsky, K. and O. Hornykiewicz. Morphine catalepsy in the rat: Relation to striatal dopamine metabolism. *Eur. J. Pharmac.* **19:** 119–122, 1972.
25. Lee, C. M. and P. C. L. Wong. Effects of morphine on the rat striatal dopamine metabolism. *Biochem. Pharmac.* **28:** 163–170, 1979.
26. Leysen, J., J. P. Tollenaere, M. H. J. Koch and P. Laduron. Differentiation of opiate and neuroleptic receptor binding in rat brain. *Eur. J. Pharmac.* **43:** 253–267, 1977.
27. Loh, H. H., D. A. Brase, S. Sampath-Khanna, J. B. Mar and E. L. Way. B-Endorphin in vitro inhibition of striatal dopamine release. *Nature* **264:** 567–568, 1976.
28. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
29. Moleman, P. and J. Bruinvels. Morphine-induced striatal dopamine efflux depends on the activity of nigrostriatal dopamine neurones. *Nature* **281:** 686–687, 1979.
30. Muller, P. and P. Seeman. Dopaminergic supersensitivity after neuroleptics: Time-course and specificity. *Psychopharmacology* **60:** 1–11, 1978.
31. Overstreet, D. H. and H. I. Yamamura. Receptor alterations and drug tolerance. *Life Sci.* **25:** 1865–1878, 1979.
32. Owen, F., A. J. Cross, J. L. Waddington, M. Poulter, S. J. Gamble and T. J. Crow. Dopamine-mediated behavior and ^{3}H-spiperone binding to striatal membranes in rats after nine months haloperidol administration. *Life Sci.* **26:** 55–59, 1980.
33. Pedigo, N. W., N. C. Ling, T. D. Reisine and H. I. Yamamura. Examination of des-tyrosine^1-y-endorphin activity at ^{3}H-spiroperidol binding sites in rat brain. *Life Sci.* **24:** 1645–1650, 1979.
34. Perez-Cruet, J., G. diChiara and G. L. Gessa. Accelerated synthesis of dopamine in the rat brain after methadone. *Experientia* **28:** 926, 1972.
35. Pollard, H., C. Llorens, J. J. Bonnet, J. Costentin and J. C. Schwartz. Opiate receptors on mesolimbic dopaminergic neurons. *Neurosci. Lett.* **7:** 295–299, 1977.
36. Pollard, H., C. Llorens, J. C. Schwartz, C. Gros and F. Dray. Localization of opiate receptors and enkephalins in the rat striatum in relationship with the nigrostriatal dopaminergic system: lesion studies. *Brain Res.* **151:** 392–398, 1978.
37. Puri, S. K. and H. Lal. Effect of dopaminergic stimulation or blockade on morphine-withdrawal aggression. *Psychopharmacologia* **32:** 113–120, 1973.
38. Puri, S. K., C. Reddy and H. Lal. Blockade of central dopaminergic receptors by morphine: Effect of haloperidol, apomorphine or benztropine. *Res. Communs chem. pathol. Pharmac.* **5:** 389–401, 1973.
39. Puri, S. K., T. C. Spaulding and C. R. Mantione. Dopamine antagonist binding: A significant decrease with morphine dependence in the rat striatum. *Life Sci.* **23:** 637–642, 1978.
40. Sasame, H. A., J. Perez-Cruet, G. DiChiara, A. Tagliamonte, P. Tagliamonte and G. L. Gessa. Evidence that methadone blocks dopamine receptors in the brain. *J. Neurochem.* **19:** 1953–1957, 1972.
41. Sayers, A. C., H. R. Burki, W. Ruch and H. Asper. Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesia: Effects of clozapine, haloperidol, loxapine and chlorpromazine. *Psychopharmacologia* **41:** 97–104, 1975.
42. Schechter, M. D. Lack of blockade of central dopaminergic receptors by narcotics: Comparison with chlorpromazine. *Eur. J. Pharmac.* **49:** 279–284, 1978.
43. Schwartz, J. C., H. Pollard, C. Llorens, B. Malfroy, C. Gros, Ph. Pradelles and F. Dray. Endorphins and endorphin receptors in striatum: Relationships with dopaminergic neurons. In: *Advances in Biochemical Psychopharmacology*, vol. 18, edited by E. Costa and M. Trabucchi. New York: Raven Press, 1978, pp. 245–264.
44. Seeger, T. F. and K. R. Carlson. Specific inhibition of d-amphetamine but not apomorphine-induced stereotypy by morphine. *Soc. Neurosci. Abstr.* **6:** 312, 1980.
45. Seeger, T. F. and E. L. Gardner. Enhancement of self-stimulation behavior in rats and monkeys after chronic neuroleptic treatment: Evidence for mesolimbic supersensitivity. *Brain Res.* **175:** 49–57, 1979.
46. Seeman, P. Antischizophrenic drugs—membrane receptor sites of action. *Biochem. Pharmac.* **26:** 1741–1748, 1977.
47. Slater, P., C. Blundell and A. R. Crossman. The effects of narcotic analgesics on the turning behavior of rats with 6-hydroxydopamine-induced unilateral nigrostriatal lesions. *Neuropharmacology* **19:** 187–193, 1980.
48. Tarsy, D. and R. J. Baldessarini. Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. *Neuropharmacology* **13:** 927–940, 1974.
49. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.