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Stereotypy and Striatal Dopamine Receptor Changes with l-α-Acetylmethadol (LAAM)

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LANGLEY, A. E., J. KIRLANGITIS AND T. LEHMAN. *Stereotypy and striatal dopamine receptor changes with l-α-acetylmethadol (LAAM).* PHARMAC. BIOCHEM. BEHAV. **14**(5) 753–756, 1981.—Narcotics like morphine and methadone produce effects on striatal dopaminergic mechanisms as evidenced by changes in a stereotyped behavior induced by apomorphine and binding of radioactive ligands to dopamine (DA) receptors. LAAM may replace methadone in the treatment of narcotic addiction. We investigated the effect of LAAM on apomorphine induced stereotypy and the binding of ^{3}H-spiroperidol to dopamine receptors isolated from mouse and guinea pig striata. Chronic LAAM treatment produced a behavioral supersensitivity to apomorphine. The biochemical expression of the behavioral effect was a significant increase in the number of dopamine receptor binding sites in mouse and guinea pig striata. These effects can be due to either a direct chronic blockade of postsynaptic dopamine receptors or stimulation of a presynaptic receptor which acts to reduce the amount of dopamine released during nerve activity. Work is in progress to determine the mechanism of this effect.

Alphacetylmethadol (LAAM) ^{3}H-spiroperidol binding Apomorphine stereotypy Opiate receptors Dopamine receptors

RECENT research has demonstrated that narcotic analgesics can influence striatal dopaminergic activity. Morphine and methadone have been reported to block central dopamine receptors [12,13]. Immediately following termination of chronic narcotic treatment in guinea pigs, there is a supersensitivity to the behavioral effects of the dopamine agonist apomorphine [3,4]. This supersensitivity is characterized by an increase in the intensity of a stereotyped gnawing and chewing behavior elicited by apomorphine. De la Baume, *et al.* [7], reported that morphine treatment can produce a supersensitivity of postsynaptic receptors by acting on presynaptic opiate receptors on the dopaminergic nerve terminal. The increase in postsynaptic DA receptor sensitivity was demonstrated by an increase in magnitude of apomorphine-induced stereotypy and a greater number of binding sites for ^{3}H-domperidone in striatal tissue. We investigated apomorphine-induced stereotypy following chronic treatment of mice and guinea pigs with the narcotic l-alpha-acetylmethadol (LAAM) a proposed replacement for methadone in narcotic maintenance programs [1]. ^{3}H-spiroperidol was used to biochemically quantitate changes in central dopamine receptors (affinity and number of binding sites) produced by chronic LAAM treatment.

METHOD

Adult male mice, 25–30 g of the NIH/Swiss strain and male albino guinea pigs, 380–450 g, Parco-Harley Strain, were used in these experiments.

Guinea pigs were treated for 70 days with 12 mg/kg PO LAAM and mice for 30 days with 16 mg/kg PO LAAM. The doses were systematically determined using the Dixon "up-and-down" method [8] and found to be effective in suppressing withdrawal signs in morphine-dependent animals for at least 24 hours. Water was administered to control groups in each species.

l-Alpha-acetylmethadol (LAAM) was a gift of the Biomedical Research Branch, National Institute of Drug Abuse, Rockville, Maryland. Apomorphine was supplied by Merck Chemical Division, Merck and Company, Rahway, New Jersey. The ^{3}H-spiroperidol (26-39 Ci/m mol) was purchased from New England Nuclear, Boston, Massachusetts. All other chemicals were obtained from commercial sources.

Behavioral Stereotypy

Four days after the final dose of LAAM, the behavioral effects of the dopamine agonist, apomorphine were measured. The method of Nausieda, *et al.* [11] was used to challenge and score guinea pigs. Four water-dosed controls and four LAAM-treated guinea pigs were injected with a single 0.2 mg/kg, IP dose of apomorphine. Stereotypy scoring was based on two-min observation periods at fifteen-min intervals by a blind observer. A modification of the method of

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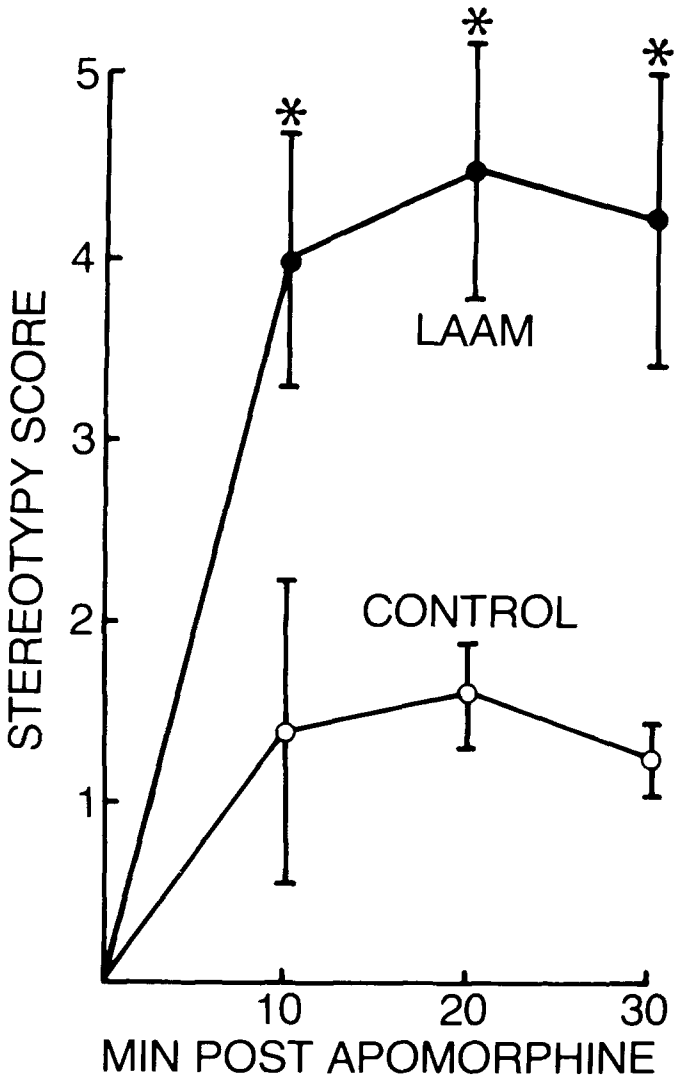

FIG. 1. Stereotypy scores in mice following a single 2.0 mg/kg IP injection of apomorphine. Control ○——○, n=9; chronic LAAM treated ●——●, n=9. *$p<0.05$, Student's t-test.

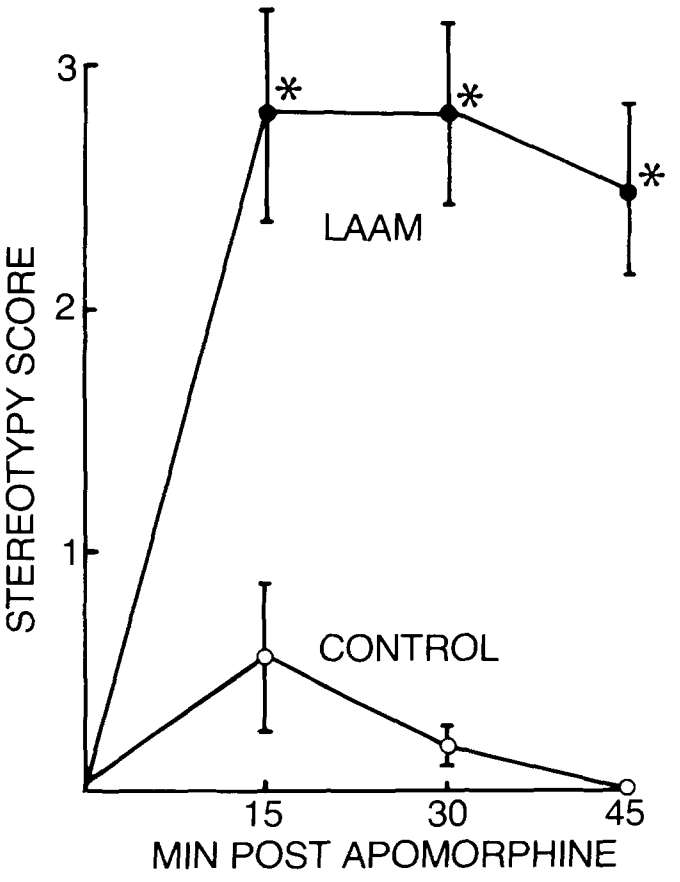

FIG. 2. Stereotypy scores in guinea pigs following a single 0.2 mg/kg IP injection of apomorphine. Control ○——○, n=4; chronic LAAM treated ●——●, n=4. *$p<0.01$, Student's t-test.

Carlson and Almasi [3] originally developed for guinea pigs was used to score mice. Nine control and nine LAAM-treated mice were injected with a single 2.0 mg/kg, IP dose of apomorphine (a dose similar to that reported by De la Baume, *et al.* [7] for provoking stereotypy in mice) and scoring was based on two-min observations at fifteen-min intervals by a blind observer. The stereotypy score at time zero refers to a two-min observation period prior to drug administration during which a baseline for stereotyped behavior was determined.

^{3}H-Spiroperidol Binding

Changes in dopamine receptors were assessed four days following termination of LAAM dosing. The striata were homogenized in 100 vol (w/v) of cold 0.5 M Tris Buffer, pH 7.7 with a Brinkman polytron. The tissue homogenates were centrifuged twice at 25,000 rpm (Sorvall OTD-2) for 15 min. The final pellet was homogenized in 150 vol of cold 0.05 M Tris Buffer containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCL, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.1 at 37°C for 10 min and returned to ice [5].

The incubation tubes contained varying concentration of ^{3}H-spiroperidol in a volume of 0.1 ml, 0.1 ml of 0.1% ascorbic acid with or without 1.0 μM d-butaclamol and 1.8 ml of tissue homogenate. Tubes were incubated for 20 min at 37°C and filtered through Whatman GF/B glass microfilters under vacuum, using the rapid filtration method of Creese, *et al.* [5]. The tubes were rinsed and the filters were washed with 3×5 ml aliquots of Tris buffer. The filters were placed into liquid scintillation vials containing Instagel (Packard) and counted on a Packard Tri-Carb scintillation counter with counting efficiencies ranging from 38–45%. The specific binding to dopaminergic receptors was estimated as the difference of binding in the absence and presence of 1 μM d-butaclamol. The kinetics of ^{3}H-spiroperidol binding was determined using Scatchard analysis. The data were also analyzed to determine the Hill coefficients which is indicative of the number of different binding sites for ^{3}H-spiroperidol [6]. A coefficient of 1.0 indicates a single binding site and a value less 0.80 is considered to be different from unity. Protein was measured by the Lowery method. All data are reported as mean ± standard error of the mean.

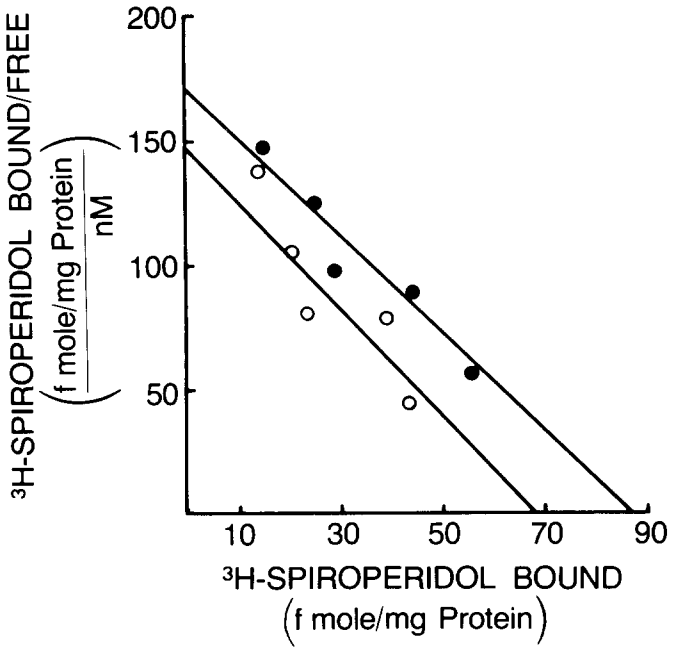

FIG. 3. Scatchard plots of specific ^{3}H-spiroperidol binding to mouse striatal membranes. Number of binding sites was significantly increased by chronic LAAM treatment ($p<0.05$, Wilcoxon-Mann-Whitney Test). The apparent affinity constant (K_D) was unchanged. Control ○——○, n=8; chronic LAAM treated ●——●, n=7. Data were subjected to linear regression analysis (correlation coefficient, r=.90 for controls and r=.97 for LAAM treated).

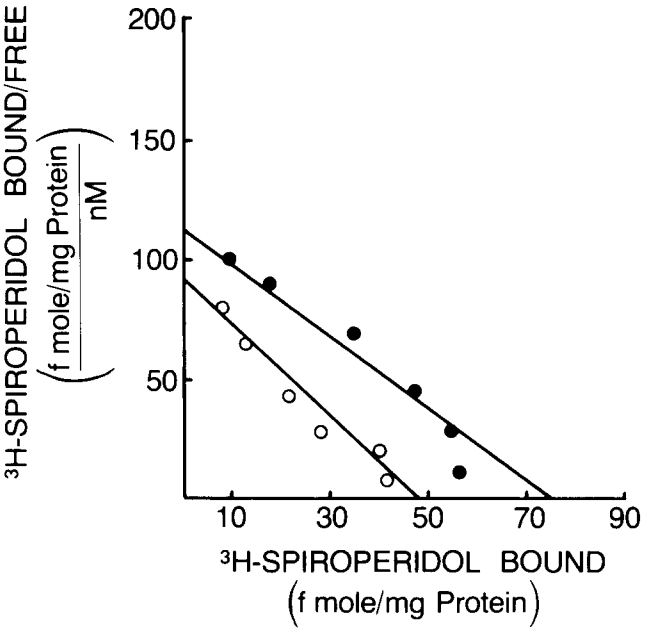

FIG. 4. Scatchard plots of specific ^{3}H-spiroperidol binding to guinea pig striatal membranes. Number of binding sites was significantly increased by chronic LAAM treatment ($p<0.05$, Wilcoxon-Mann-Whitney Test). The apparent affinity constant was unchanged. Control ○——○○, n=5; chronic LAAM treated ●——●, n=4. Data were analyzed by linear regression analysis with correlation coefficients: r=.92 for controls and r=.98 for LAAM treated.

RESULTS

Apomorphine Induced Stereotypy

Mice (Fig. 1) and guinea pigs (Fig. 2) demonstrated significantly higher stereotypy scores at all test times following a single injection of apomorphine. Guinea pigs were observed for a longer period of time in order to establish a time-course for the apomorphine effect. After 45 min control guinea pigs returned to base-line (pre-drug) activity while chronically LAAM-treated guinea pigs demonstrated a significantly elevated stereotypy score.

Dopamine Receptor Binding

Analysis of the binding data support a single binding site for ^{3}H-spiroperidol. Striatal tissue from mice demonstrated Hill coefficients of 0.90±0.03 for control, 0.96±0.02 for LAAM-treated animals. The guinea pig striata had Hill coefficients of 0.96±0.03 for controls, and 0.84±0.05 for LAAM-treated animals. Scatchard analysis of ^{3}H-spiroperidol binding to striatal dopamine receptors from mouse brain (Fig. 3) shows no change in the apparent affinity constant (K_D) following chronic LAAM treatment (control = 0.48±0.05 nM vs LAAM = 0.55±0.07 nM). The total number of binding sites is significantly greater in the striata from LAAM treated mice (87.3±3.1 fmole/mg protein) than vehicle-treated controls (68.6±4.8 fmole/mg protein). Striatal tissue from guinea pigs demonstrated similar effects. The total number of binding sites in guinea pig brain was significantly greater in LAAM-treated animals (75.5±3.6 fmole/mg protein) than the controls (47.6±5.6 fmole/mg protein) while the apparent affinity constant was unchanged by chronic LAAM treatment (LAAM = 0.68±0.05 nM vs control = 0.54±0.10 nM.

DISCUSSION

A number of reports have indicated that long-term treatment with narcotics like morphine and methadone can produce changes in the central dopaminergic system similar to those produced by chronic neuroleptic therapy [2, 3, 4, 7, 10]. Because LAAM is being explored as a replacement for methadone in maintenance programs for recovering narcotic addicts [1], its potential effects on striatal dopaminergic mechanisms needs to be explored. By measuring changes in central homovanillic acid levels and dopamine turnover in basal ganglia Sasame and Perez-Cruet [13] concluded that methadone, like the neuroleptics, blocked dopamine receptors. A later report by Kuschinsky and Hornykiewicz [9] suggested that morphine's effect on the dopaminergic system was via a presynaptic mechanism. The data reported here show that LAAM possesses the dopaminergic altering activity of other narcotic analgesics. The behavioral response to apomorphine is augmented by chronic LAAM treatment. The increased responsiveness to apomorphine is reflected in a greater than normal number of striatal dopamine binding sites. These data do not resolve the exact site of action of LAAM which can be due to either direct chronic blockade of postsynaptic dopamine receptors or chronic stimulation of a presynaptic receptor which acts as a negative modulator of dopamine release. Either situation would lead to postsynaptic dopamine receptor supersensitivity.

REFERENCES

1. Blaine, J. and P. Renault. LAAM alternative to methadone. *N.I.D.A. Research Monograph Series* **8:** 1–9, 1976.
2. Carenzi, A., D. L. Cheney, E. Costa, A. Guidotti and G. Racagni. Action of opiates, antipsychotics, amphetamine and apomorphine on dopamine receptors in rat striatum: in vivo changes of 3′, 5′-cyclic AMP content and acetylcholine turnover rate. *Neuropharmacology* **14:** 927–939, 1975.
3. Carlson, K. R. and J. Almasi. Behavioral supersensitivity to apomorphine following chronic narcotic treatment in the guinea pig. *Psychopharmacology* **57:** 273–277, 1978.
4. Carlson, K. R. and J. Almasi. Time course of dopaminergic hypersensitivity following chronic narcotic treatment. *Pharmac. Biochem. Behav.* **11:** 283–287, 1979.
5. Creese, I., R. Schneider and S. H. Snyder. ^{3}H-Spiroperidol labels dopamine receptors in pituitary and brain. *Eur. J. Pharmac.* **46:** 377–381, 1977.
6. Dahlquist, F. W. Meaning of Scatchard and Hill Plots. *Meth. Enzym.* **48:** 270–299, 1978.
7. 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 treatments. *Life Sci.* **24:** 2333–2342, 1979.
8. Dixon, W. J. The up-and-down method for small samples. *J. Am. statist. Ass.* **60:** 967–978, 1965.
9. Kuschinsky, K. and O. Hornykiewicz. Effects of morphine on striatal dopamine metabolism: Possible mechanism of its opposite effect on locomotor activity in rats and mice. *Eur. J. Pharmac.* **26:** 41–50, 1974.
10. Lal, H. Narcotic dependence, narcotic action and dopamine receptors. *Life Sci.* **17:** 483–496, 1976.
11. Nausieda, P. A., W. J. Weiner, D. J. Kanapa and H. L. Klawans. Bromocriptine-induced behavioral hypersensitivity: implications for the therapy of parkinsonism. *Neurology* **28:** 1183–1188, 1978.
12. 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.
13. Sasame, H. A. and J. Perez-Cruet. Evidence that methadone blocks dopamine receptors in the brain. *J. Neurochem.* **19:** 1953–1957, 1972.