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# STRESS-ACCENTUATION OF THE LSD-INDUCED DISAGGREGATION OF BRAIN POLYSOMES

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Summary

The application of three types of stress; restraint, food deprivation or epinephrine injection markedly accentuated the disaggregation of rabbit brain polysomes to monosomes induced by LSD (25 µg/kg) whereas no shift of polysomes to monosomes was found with any of the stress treatments alone. LSD when administered intravenously at a very low dose of 1 µg/kg and combined with the restraint procedure produced a massive brain polysome shift. LSD alone at this dosage did not induce a disaggregation of polysomes. Elevations in plasma corticosteroid levels relative to control were found following LSD administration with or without the stressing procedures. LSD and certain elements of environment and physiological arousal appear to have a synergistic effect on disrupting the protein synthesis apparatus of brain.

Previously we have reported that the intravenous administration of D-lysergic acid diethylamide (LSD) to young rabbits induces a transient disaggregation of brain polysomes which is mediated by the interaction of the drug with neurotransmitter receptors (13-15). The effect is brain specific and the degree of polysome shift increases with age from 3 week old rabbits to adults (13, 14). Since this LSD-induced polysome shift was shown to be altered by holding-cage environment, pre-LSD sedation and post-LSD handling (14), it appeared that certain elements of environment and physiological arousal were involved in the macromolecular effect of the drug on the protein synthesis apparatus of brain.

In the present paper we show that the application of stress following drug administration greatly accentuates the LSD-induced disaggregation of brain polysomes. Doses as low as 1 µg/kg when combined with stress can produce a massive shift of brain polysomes to monosomes. Low doses of LSD or stress when administered independently have no effect on brain polysomes. LSD also produced a stress-like response in rabbits as indicated by a rise in plasma corticosteroids.

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## Material and Methods

### Drug Administration and Stress Treatments

All experiments were carried out on young male rabbits (1.2-1.6 kg) kept on a 7 a.m.-7 p.m. light-dark cycle, at 22°C and fed ad lib. D-LSD (Sandoz Pharmaceuticals, Switzerland) obtained through the Department of National Health and Welfare, Ottawa, Canada was dissolved in 0.9% NaCl and injected into a marginal ear vein between 9:00 and 10:30 a.m. Control animals received an appropriate volume of physiological saline. After injection the rabbits were placed in an open-top box exposed to laboratory lighting and sounds and left without food or water until despatched by cervical dislocation. Three types of stress were utilized in these experiments. The first was restraint and handling, in which the animal's front and hind limbs were taped loosely so as not to interfere with circulation yet sufficient to impede normal movement. The animals were rolled onto their backs every 5-10 min. Other animals were given 25 µg/kg of epinephrine bitartrate (Sigma) concomitant with LSD or deprived of food for 24 hr prior to LSD injection.

### Polysome Isolation and Analysis

The brains were quickly removed and placed in cold 0.25 M sucrose buffered with 50 mM Tris-HCl pH 7.5 @ 4°C, 25 mM KCl, 5 mM $MgCl_2$ and 1 mM dithiothreitol (Buffer A). The brain was dissected into the cerebral hemispheres, cerebellum and the remaining stem region. Polysomes were isolated by a slight modification of the method of Holbrook and Brown (13) which was introduced to improve ribosome recovery. The detergent-treated post-mitochondrial supernatant was layered over 5 ml of 1.5 M sucrose rather than 2.0 M sucrose prior to centrifugation at 50,000 rev/min for 3 hr in a Beckman L2-65B ultracentrifuge. The polysomes were analyzed by sucrose gradient centrifugation as described previously (13). The area under the polysome and monosome region was determined by planimetry. The statistical significances have been calculated according to the Student's t-test.

### Corticosteroid Assay

Rabbit plasma corticosteroids were assayed by a modification of the competitive protein binding technique described by Murphy (22). Rabbit plasma samples (100 µl) were extracted with 1 ml of methylene chloride. The methylene chloride phase was removed and evaporated in a 40°C water bath. One ml aliquots of 2% ethanol: distilled water (v/v) were added to each of the dried sample tubes. Corticosterone standards ranging from 2.5 to 25 ng were made up in the same medium. One ml of the [$^3$H]-corticosteroid binding globulin (5% rabbit plasma containing 5 µCi of [$^3$H]-corticosterone @ 54.5 Ci/mmol; New England Nuclear) was added to each tube and incubated for 5 min. The tubes were cooled in an ice bath for 30 min followed by the addition of 160 mg of Florisil (Fisher) to each tube and vortexed vigorously for 15 sec. The tubes were returned to the ice bath for 1 hr and 500 µl of the supernatants were then counted in 10 ml of toluene scintillation fluor containing Beckman Bio-Solv BB-3 solubilizer.

## Results

Since our previous studies have indicated that the degree of LSD-induced brain polysome disaggregation is altered by elements of environment and physiological arousal (14) we decided to examine the effect of administering stress immediately following drug injection. Three forms of stress were employed; restraint and handling; food deprivation for 24 hr and epinephrine injection. As seen in Figure 1 the application of these three forms of stress following the intravenous injection of LSD (25 µg/kg) accentuated the shift of brain polysomes to monosomes relative to that observed with LSD alone. The non-hallucinogenic analogue of LSD, 2-bromo-LSD (200 µg/kg) did not disaggregate polysomes with or without stress. A quantitative representation of these results was obtained by calculating the percentage of monosomes produced following LSD or LSD plus stress (Table 1). LSD administered at a dose of 25 µg/kg produced a 40-60% increase in monosomes over controls in all three brain regions. The application of restraint following drug administration significantly accentuated this response, since a 150-165% increase in monosomes was observed relative to saline control values. An augmented polysome shift was also noted with both LSD plus epinephrine and LSD plus the food deprivation treatment (i.e. a 100-120% increase in monosomes over saline controls). No significant shift of brain polysomes to monosomes was observed with any of the stress treatments alone. As mentioned earlier, the non-psychotropic drug, 2-bromo-LSD did not produce any increase in the percentage of monosomes over control with or without restraint.

Since the combination of stress with a moderate dose of LSD (25 µg/kg) resulted in an accentuated polysome shift, it was of interest to determine the effect of restraint on animals which had been given reduced dosages of LSD. As shown in Table 2 LSD administered at a dose of 10 µg/kg, followed by restraint, produced a 185% increase in cerebral hemisphere monosomes expressed over the saline control values. Slightly lower responses were observed in the brain stem and cerebellum. LSD alone at this dose produced only a 30-50% increase in monosomes. An LSD dosage of 1 µg/kg when combined with the restraint procedure showed a 100-125% increase in monosomes over controls. LSD alone at this dose (1 µg/kg) did not produce any detectable effect on brain polysomes.

Our previous results have shown that LSD induces a disaggregation of brain polysomes by a non-RNase mechanism (13, 14). For example, the monosome pool produced following LSD administration is dissociable into ribosomal subunits by high salt (500 mM KCl). Monosomes produced after treatment of polysomes with exogenous RNase do not dissociate under these salt conditions. This technique was utilized in the present study to determine whether stress accentuation of the LSD-induced disaggregation of polysomes was due to RNase activation. In the presence of high salt the elevated pool of monosomes which is produced after LSD (1 µg/kg) plus restraint was found to dissociate into 60S and 40S ribosomal subunits (Figure 2). The same result with respect to monosome dissociability was observed with other forms of stress (epinephrine injection and food deprivation) in combination with LSD (25 µg/kg). The increase in monosomes following the application of restraint plus LSD is therefore likely produced via a non-RNase

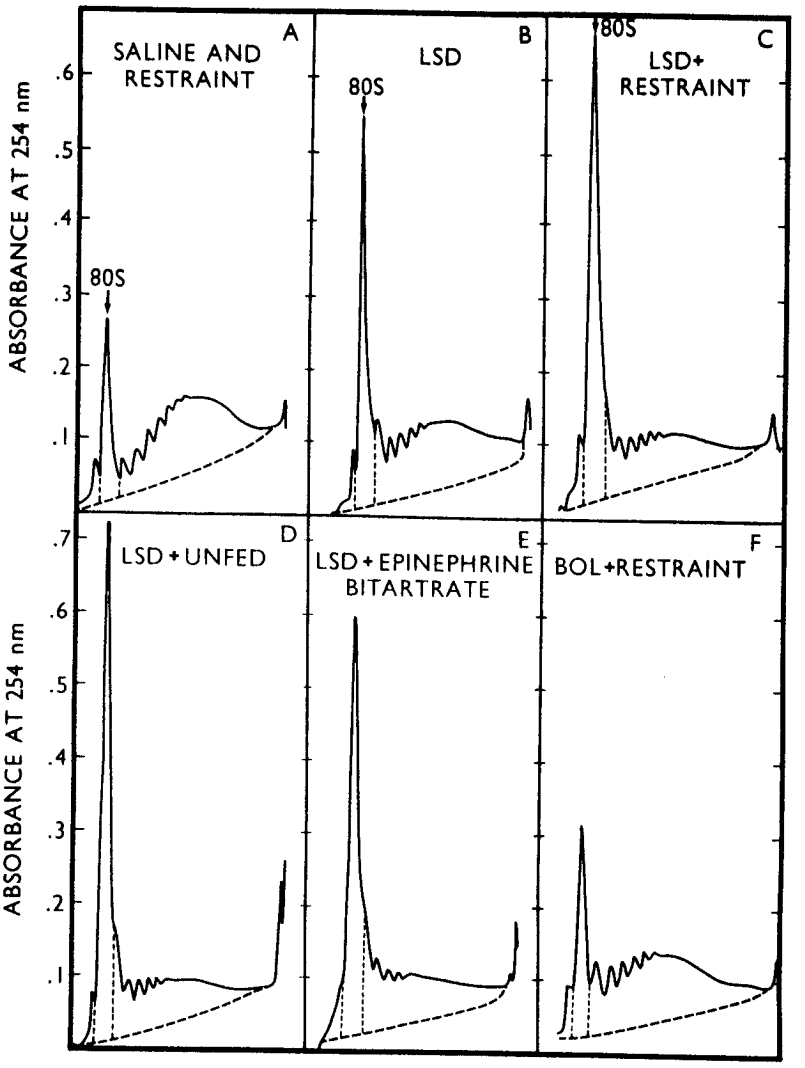


FIG. 1

Stress accentuation of the disaggregation of brain polysomes induced by LSD *in vivo*. LSD (25 µg/kg), BOL (200 µg/kg) or an appropriate volume of 0.9% NaCl were administered intravenously to young rabbits in conjunction with one of the stress treatments outlined in Materials and Methods. Polysomes from cerebral hemispheres were isolated 1 hr after drug injection and analyzed on 15-45% sucrose density gradients following centrifugation for 75 min in a Beckman SW-41 rotor at 40,000 rpm @ 4°C (13).

TABLE 1

| Treatment | Trials | Brain Region % Monosomes/Total Ribosomes Cerebral Hemispheres | Stem | Cerebellum |
|---|---|---|---|---|
| Saline | 9 | 21.8±2.6 | 23.3±1.8 | 19.9±2.3 |
| LSD | 8 | 36.3±3.4 | 33.3±3.4 | 28.6±4.4 |
| LSD & Restraint | 6 | 54.5±4.6* | 55.6±4.3* | 53.0±5.2* |
| Saline & Restraint | 8 | 21.1±2.5 | 21.9±3.4 | 19.3±2.1 |
| LSD & Epi. Bit. | 4 | 49.0±4.1* | 47.5±1.7* | 39.0±4.1* |
| Saline & Epi. Bit. | 2 | 23.0±0.0 | 24.5±0.5 | 22.5±0.5 |
| LSD & Unfed | 4 | 47.8±9.1* | 46.0±6.7* | 44.1±6.5* |
| Saline & Unfed | 2 | 20.5±4.9 | 23.0±0.0 | 17.5±2.3 |
| BOL | 3 | 19.6±2.5 | 20.3±3.1 | 18.7±1.5 |
| BOL & Restraint | 4 | 20.3±2.8 | 21.5±1.9 | 19.5±1.9 |

Quantitation of the accentuation of the LSD-induced disaggregation of brain polysomes by stress. Animals were given either LSD (25 µg/kg) or an appropriate volume of 0.9% NaCl (i.v.) plus one of the stress treatments as outlined in the Materials & Methods. BOL was administered at a dosage of 200 µg/kg. After 1 hr the animals were despatched and polysomes were isolated and % monosomes determined by analysis on sucrose gradients (13). The level of statistical significance ($p<0.01$) relative to the LSD (25 µg/kg) value is indicated with an asterisk. (Epi. Bit., epinephrine bitartrate).

TABLE 2

| Treatment | Trials | Brain Region % Monosomes/Total Ribosomes Cerebral Hemispheres | Stem | Cerebellum |
|---|---|---|---|---|
| Saline & Restraint | 8 | 21.1±2.5 | 21.9±3.4 | 19.3±2.1 |
| LSD 10 µg/kg | 5 | 30.6±8.2 | 31.6±8.1 | 25.2±5.0 |
| LSD 10 µg/kg & Restraint | 4 | 50.3±3.8* | 46.8±3.4* | 41.0±5.0* |
| LSD 1 µg/kg | 4 | 21.8±2.5 | 21.8±3.9 | 19.5±1.7 |
| LSD 1 µg/kg & Restraint | 6 | 45.3±1.7* | 44.8±2.9* | 43.2±5.2* |

Restraint-accentuated disaggregation of brain polysomes at reduced dosages of LSD. Animals were given LSD intravenously at the dose indicated and then subjected to the restraint procedure for 1 hr. Brain polysomes were then isolated and analyzed on sucrose gradients (13). Levels of statistical significance ($p<0.01$) relative to the same dose of LSD without restraints are indicated with an asterisk.

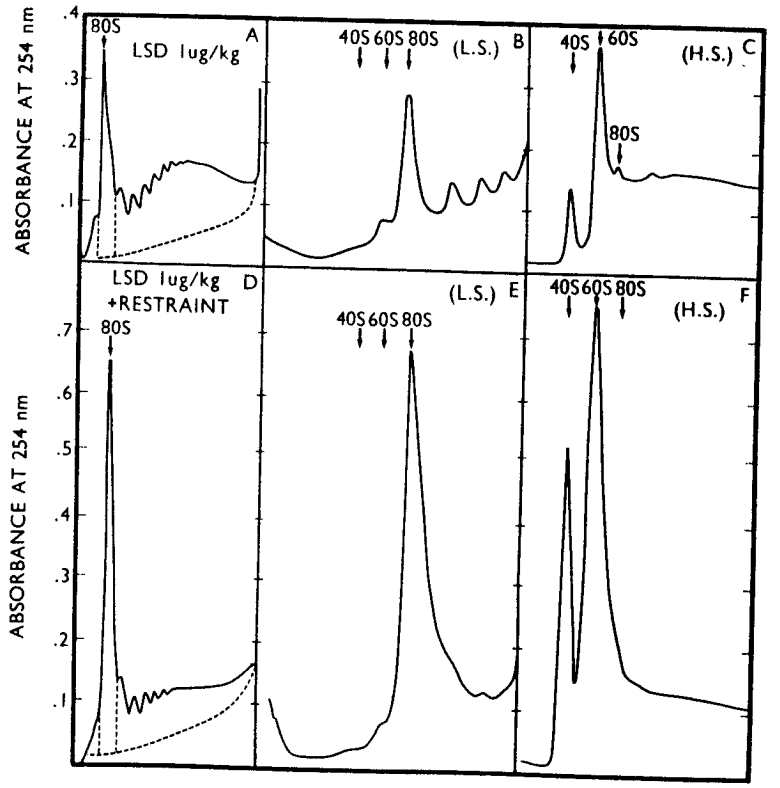


FIG. 2

Dissociation of monosome peaks to ribosomal subunits by high salt treatment. Cerebral hemisphere polysomes were isolated from young rabbits 1 hr after intravenous injection of LSD at 1 µg/kg (panels A-C) or 1 µg/kg LSD plus restraint (panels D-F). Aliquots of polysomes were analyzed on 15-45% sucrose gradients containing buffer A with 25 mM KCl (L.S.=low salt) or 500 mM KCl (H.S.=high salt). In order to display ribosomal subunits the gradients were centrifuged for 3.5 hr under conditions given in Fig. 1. For comparison, complete polysome profiles are shown in panels A and D (centrifugation for 75 min in low salt).

mechanism.

Plasma corticosteroid levels were analyzed to determine whether LSD and the three types of applied stress produced a measurable stress-like response. The administration of LSD at a dose of 25 µg/kg did not significantly elevate plasma corticosteroids after 1 hr whereas higher doses of LSD (50 and 100 µg/kg) resulted in a 3-fold increase over control values (Table 3). LSD (25 µg/kg) in combination with each of the stressing procedures significantly elevated the level of plasma corticosteroids relative to controls. Each of these treatments yielded values 2-3-fold higher than that observed with 25 µg/kg of LSD alone. Restraint without injection of LSD resulted in an increase in plasma corticosteroids, however, and as shown previously no shift in brain polysomes was observed.

TABLE 3

| Treatment | Trials | Plasma Corticosteroids (µg/100 ml) |
|---|---|---|
| Saline | 7 | 2.4±0.6 |
| LSD 25 µg/kg | 5 | 3.1±0.6 |
| LSD 50 µg/kg | 4 | 6.8±1.0* |
| LSD 100 µg/kg | 4 | 6.9±0.6* |
| Saline & Restraint | 4 | 8.0±1.5* |
| LSD 25 µg/kg & Restraint | 6 | 9.6±3.3* |
| LSD 25 µg/kg & Epi. Bit | 5 | 5.0±1.1* |
| LSD 25 µg/kg & Unfed | 4 | 5.6±1.5* |

Effect of LSD and stress on the level of plasma corticosteroids. Rabbits were sacrificed 1 hr after drug or saline administration and trunk blood was collected after decapitation. Plasma corticosteroids were assayed as outlined in Materials and Methods. Levels of statistical significance ($p<0.01$) relative to the saline control value are indicated with an asterisk.

## Discussion

Previously we have shown, utilizing neurotransmitter receptor blocking agents, that the LSD-induced disaggregation of brain polysomes is a receptor-mediated event (15) and that the degree of polysome shift is dependent on environment and post-LSD handling (14). A dependence of LSD action on environment and physiological arousal has also been reported by others. Boakes et al. (5) have shown that activation of EEG's by LSD is markedly dependent on environmental factors while King et al. (17) found that the duration and intensity of the LSD effect during reversal learning was highly dependent on a 'home' versus 'strange' environment. Low doses of LSD was also found to have an excitatory effect on the acoustic startle reflex in the rat (8) and aggravate the stress-induced reduction in brain and blood glutathione levels (33). A

synergistic interaction of LSD and stress is evident in our present results which shows that physiological stress applied in combination with low doses of LSD, significantly accentuates the shift of brain polysomes to monosomes relative to that observed with LSD alone.

All three forms of stress; restraint, food deprivation and epinephrine injection accentuated the LSD-induced disaggregation of polysomes. Each of the above treatments did in fact produce a measurable stress-like response as evidenced by an elevation in plasma corticosteroids. LSD alone at doses higher than 25 µg/kg also induced a significant elevation in plasma corticosteroids. This is in agreement with previous studies which show an increase in plasma corticosteroid levels in humans and rats after LSD administration (12, 27). Therefore LSD may induce ACTH release by some as yet unknown mechanism. LSD may exert both antagonistic and agonistic actions on 5-HT receptors and therefore may interfere with the serotonergic or noradrenergic mechanisms which have been implicated in the regulation of the hypothalamic-pituitary-adrenal system (23, 24, 25, 28).

The observed polysome disaggregation was dependent on the administration of LSD since none of the stressing procedures could independently induce a shift. Furthermore this phenomenon required the psychoactive form of LSD, as 2-bromo-LSD, the non-psychotropic derivative of LSD which also binds to the same neural receptors as LSD (6, 19) could not induce a polysome shift with or without stress. The administration of LSD at a dosage as low as 1 µg/kg was sufficient to induce a massive brain polysome shift when combined with the restraining procedure. This dosage is well within levels utilized by humans (16, 27). In contrast, very high dosages of other agents such as phenylalanine, L-dopa, 5-HTP and D-amphetamine are required to induce disaggregation of brain polysomes (1, 20, 30, 35).

We have previously shown that nerve conductivity is a requirement for the LSD-induced polysome shift since depression of nerve conductivity by anaesthetic doses of ethanol, pentobarbitol or diphenylhydantoin inhibits this response (15). Also low doses of LSD administered to rabbits produce definite EEG arousal patterns indicative of elevated neuronal activity (10, 15). Furthermore the stress procedures utilized in the present study may also activate channels of sensory feedback since increases in the activity of noradrenergic neurons have been consistently found during acute exposures to a wide variety of stresses such as foot-shock, immobilization, cold ambient temperatures, forced running and inhibition of glucose metabolism (3, 7, 11, 18, 21, 29, 31). These observations raise the possibility that an elevated level of neuronal activity in certain brain regions after LSD or LSD plus stress may be involved in the disaggregation of brain polysomes.

Our previous results have demonstrated that the LSD-induced disaggregation of polysomes is likely produced via a non-RNase mechanism since the large monosome pool produced after administration of LSD *in vivo* was almost completely dissociable into subunits by high salt (13). Furthermore measurements on the association of tRNA with ribosomes were interpreted as revealing that the increased pool of monosomes after LSD was produced by normal

run-off (14). Preliminary evidence suggests that this metabolic lesion is due to inhibited reinitiation and/or elongation (14). The demonstration that the monosomes produced by LSD plus stress are dissociable into subunits in high salt suggests that the same mechanism is operative and that the accentuated shift is not the result of an elevation in RNase activity (4).

The heightened neuronal activity resulting from LSD and stress may produce a depletion of energy reserves which could result in a transient decrease in protein synthesis (2, 9, 34). Since LSD is known to elevate levels of cAMP in brain (32), another possible mode of inducing brain polysome disaggregation is through the activation of cAMP-dependent protein kinases which may phosphorylate specific initiation and/or elongation factors. Alterations in cAMP levels have been suggested as a modulating agent in the action of corticotrophin:$ACTH_{1-24}$ on protein synthesis (26).

The results presented in this study clearly show a synergistic relationship between LSD and stress. Since LSD may stimulate selective central serotonergic or dopaminergic sites which in turn evoke heightened physiological responses with environmental dependency, sensory feedback may alter synaptic activity and ultimately protein synthesis. We are presently utilizing receptor blockers and neurotransmitter depleting agents in an attempt to dissect out the various neural components involved in this metabolic lesion.

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## References

1. K. AOKI and F. SIEGEL, Science N.Y. 168 129-130 (1970).
2. G. S. BENNETT and G. EDELMAN, Science N.Y. 163 393-395 (1969).
3. E. BLISS, J. AILON and J. ZWANZIGER, J. Pharmacol exp. Ther. 164 122-134 (1968).
4. G. BLOBEL and D. SABITINI, Proc. Natn. Acad. Sci. U.S.A. 68 390-394 (1971).
5. R. BOAKES, P. BRADELY, I. BRIGGS and A. DRAY, Br. J. Pharmacol. 40 202-218 (1970).
6. D. R. BURT, I. CREESE and S. H. SNYDER, Mol. Pharmacol. 12 631-638 (1976).
7. H. CORRODI, K. FUXE and T. HOKFELT, Life Sci. 7 107-112 (1968).
8. M. DAVIS, D. GALLAGHER and G. AGHAJANIAN, Life Sci. 20 1249-1258 (1977).
9. A. DUNN, Brain Res. 61 442-445 (1973).
10. L. GOLDSTEIN, in "LSD-A Total Study" (D. V. Sankar, ed.) 395-429 P.J.D. Publ. Ltd. Westbury N.Y. (1975).
11. R. GORDON, S. SPECTOR, A. SJOERDSMA and S. UNDENFRIEND, J. Pharmacol. exp. Ther. 153, 440-447 (1966).
12. A. HALARIS, D. X. FREEDMAN and V. FANG, Life Sci. 17 1467-1472 (1976).
13. L. HOLBROOK and I. R. BROWN, J. Neurochem. 27 77-82 (1976).
14. L. HOLBROOK and I. R. BROWN, J. Neurochem. 29 461-467 (1977).
15. L. HOLBROOK and I. R. BROWN, Life Sci. 21 1037-1044 (1977).

16. H. KALANT and J. KHANNA in "Principles of Medical Pharmacology" (P. Seeman and E. Sellers, ed.) 279-284 University of Toronto Press, Toronto (1975).
17. A. KING, I. MARTIN and D. ARABELLA-MELVILLE, Br. J. Pharmacol. 52 419-426 (1974).
18. J. KORF, G. AGHAJANIAN and R. ROTH, Neuropharmacol. 12 933-938 (1973).
19. R.A. LOVELL and D.X. FREEDMAN, Mol.Pharmacol. 12 620-630 (1976).
20. M. MOSKOWITZ, B. WEISS, L. LYTLE, H. MUNRO and R. WURTMAN, Proc. Natn. Acad. Sci. U.S.A. 72 834-836 (1975).
21. J. MUSACCHIO, L. JULOU, S. KETY and J. GLOWINSKI, Proc. Natn. Acad. Sci. U.S.A. 63 1117-1119 (1969).
22. B. MURPHY, J. Clin. Endocr. 27 973-990 (1967).
23. E. NAUMENKO, Brain Res. 11 1-10 (1968).
24. E. NAUMENKO, Neuroendocrinol. 5 81-88 (1969).
25. N. POPOVA, L. MASLOVA and E. NAUMENKO, Brain Res. 47 61-67 (1972).
26. M. REITH, P. SCHOTMAN, W. GISPEN and D. de WIED, Trends Biochem. Sci. 2 56-58 (1977).
27. G. V. ROSSI, Amer. J. Pharm. 2 38-44 (1971).
28. J. STOLK, J. BARCHAS, M. GOLDSTEIN, W. BOGGAN and D. FREEDMAN J. Pharmacol. exp. Ther. 189 42-50 (1974).
29. E. STONE, J. Physiol. 224 165-169 (1973).
30. F. TAUB and T. C. JOHNSON, Biochem. J. 151 173-180 (1975).
31. A. THIERRY, F. JAVOY, J. GLOWINSKI and S. KETY, J. Pharmacol. exp. Ther. 163 163-171 (1968).
32. P. UZONOV and B. WEISS, in "Advances in Cyclic Nucleotide Research" (R. Greengard, ed.) Vol. 1 435-452 Raven Press, N.Y. (1972).
33. R. VARMA, K. KHUTETA and P. DANDIYA, Psychopharmacol. 12 170-175 (1968).
34. C. WASTERLAIN, J. Neurochem. 23 253-259 (1974).
35. B. F. WEISS, J. LEIBSHUTZ, R. J. WURTMAN and H. N. MUNRO, J. Neurochem. 24 1191-1195 (1975).