Pharmacology Biochemistry & Behavior, Vol. 21, pp. 633–639, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

The Effect of the Beta-adrenergic Receptor Antagonist, Propranolol, on the Cerebral Spread of a Memory Trace in Mice

LOUIS B. FLEXNER,[1] ALLEN C. CHURCH,* JOSEFA B. FLEXNER AND THOMAS C. RAINBOW†

*Departments of Anatomy and †Pharmacology and Institute of Neurological Sciences University of Pennsylvania, Philadelphia, PA 19104 and *National Institute of Mental Health, St. Elizabeth's Hospital, Washington, DC 20032*

Received 17 October 1983

FLEXNER, L. B., A. C. CHURCH, J. B. FLEXNER AND T. C. RAINBOW. *The effect of the beta-adrenergic receptor antagonist, propranolol, on the cerebral spread of a memory trace in mice.* PHARMACOL BIOCHEM BEHAV 21(4) 633–639, 1984.—Bi-temporal injections of puromycin that primarily affect the hippocampal-entorhinal areas consistently induce amnesia of aversive maze-learning in mice for 3 days after training but are consistently ineffective if given 6 or more days after training. At these later times, additional puromycin sites covering widespread areas of the forebrain are necessary to induce amnesia. Consistent with other evidence, these observations are interpreted to indicate that the locus of the memory trace becomes more widespread during the 6-day period. A single subcutaneous injection of (−)-propranolol (50 μg/kg) given either before or 2 days after training suppressed engram spread for 60–90 days, at which time engram spread spontaneously occurred. This effect of propranolol was stereospecific. Suppression of engram spread persisted for a prolonged period in spite of the rapid recovery (about 4 hr), following treatment, of the normal level of specific binding of ^{3}H-dihydroalprenolol in membrane preparations of the cerebral hemispheres and of ^{125}I-pindolol in selected areas of the forebrain, diencephalon and brainstem.

Beta-adrenergic antagonist Propranolol Puromycin Memory spread Quantitative autoradiography

THE forebrain distribution of the engram of shock-motivated Y-maze learning in mice can be followed by exploiting the amnesic effects of intracerebral injections of puromycin. Bitemporal injections [10] of puromycin (90 μg/injection; total 180 μg) that primarily affect the hippocampal-entorhinal areas [8,9] consistently cause amnesia of aversive maze-learning in mice for 3 days after training but are consistently ineffective if given 6 or more days after training [10,22]. At these later times, amnesia is obtained only by making 6 widespread injections (30 μg/injection; total 180 μg) that affect, in addition to the temporal lobe areas, all of the neocortex and to a lesser degree the thalamus and corpus striatum [7]. Several observations support the view that in these experiments the effective amnesic agent is peptidyl-puromycin [4, 5, 11].

Our behavioral findings, consistent with other experimental [4, 10, 22, 27] as well as clinical observations [2, 14, 20, 25, 29, 30] which we have briefly reviewed [6], are interpreted to indicate that the locus of the engram spreads from the hippocampal-entorhinal areas to widespread forebrain areas within a 6-day period, and thus they have provided a method that tests for the presence of a widely distributed engram. This interpretation and use of the term, widespread engram, does not attempt to distinguish between two possibilities: (a) that development of the independently effective forebrain engram is entirely dependent upon temporal lobe-dependent programming of forebrain sites or (b) that as a result of training, a widespread forebrain memory trace is present but that initially the hippocampal area has a special role in processing information which, with time, is also acquired by the forebrain. In our experiments, the induction of amnesia by bitemporal injections of puromycin is taken to mean that the engram (i.e., independently effective engram) is confined to the temporal lobes; the absence of amnesia following this treatment is taken to mean that the widespread engram is present.

Using this approach, we have reported [6] that an intraperitoneal injection of inhibitors of dopamine β-hydroxylase, given on days 1–3 after training, delayed the spread of memory from the normal period of 6 days to a period of about one month. This treatment, repeated 5 times with intervals of 15 days between treatments, blocked engram spread for about 3 months; surprisingly, in spite of this long period of suppression, spread was again evident about one month after the last treatment. Treatment with the inhibitors for 3 consecutive days before training led to results similar to those found when this treatment followed training. In these cases, engram spread required about one month with an interval of 21 days between treatment and training which was followed 10 days later by injection of puromycin. These findings indi-

[1]Requests for reprints should be addressed to L. B. Flexner, Department of Anatomy/G3, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

cate that the suppression of engram spread caused by treatment with the inhibitors both before and after training is a spontaneously reversible process.

Following these results with inhibitors of norepinephrine synthesis, we found in preliminary experiments that engram spread of Y-maze learning could also be blocked by post-training treatment with the β-adrenergic receptor antagonist, (±)-propranolol. The experiments reported here were designed: (1) To evaluate the effect of dosage of (−)-propranolol, administered post-training on the spread of memory; (2) To test the stereospecificity of the effect of propranolol on the spread of memory; (3) To test the spontaneous reversibility of the suppression of engram spread by (−)-propranolol; (4) To determine the effect of pretraining treatment with (−)-propranolol on the spread of memory; and (5) To estimate the degree and duration of cerebral receptor blockade following administration of (−)-propranolol.

METHOD

Animals and Behavioral Procedures

Male and female Swiss-Webster mice (35–40 g) from our closed colony were housed 4 to a cage at room temperature with free access to water and standard laboratory chow. After random selection, the mice were placed in individual cages the day before use. They were trained in a single session in a Y-maze, previously described [8], to a criterion of 9 out of 10 correct responses. Intermittent foot-shock, manually applied (0.2–0.4 mA from a DC source; 2 sec on, 2 sec off), was given for failure to move from the stem of the Y within 5 sec and for errors of left-right discrimination. Shock was adjusted with individual mice to the minimal level (not less than 0.2 mA) that produced the desired behavioral response. After entering the correct arm of the maze and remaining there for 10 sec, the mouse was allowed to climb up a ladder and was returned to its home cage for 30 sec before starting the next trial. The same procedure was used 8–10 days after puromycin in tests for retention of memory of the training experience.

Total errors were the sum of failures to make a choice within 5 sec and of incorrect choices, i.e., all mistakes were added until, in 10 consecutive runs in the maze, the mouse had performed correctly in 9 of them. Memory was evaluated in the retention tests in terms of the percentage savings of errors. This percentage was calculated by subtracting the number of errors to criterion in the retention tests from the number to criterion in training, dividing by the number in training and multiplying by 100. Negative savings were scored as zero.

The intracerebral injection technique has been described [10]. Bitemporal injections were made at a depth of 2 mm from the surface of the skull through needle holes in the skull located just above the angle between the caudal sutures of the parietal bones and the origins of the temporal muscles. Each injection of puromycin (ICN Pharmaceuticals) contained 90 μg of the hydrochloride dissolved in water and neutralized with NaOH. Each injection required 2 sec for delivery; the interval between injections was less than a minute.

(±)-Propranolol (Sigma) as well as (−)- and (+)-propranolol (kindly donated by Ayerst Laboratories) were dissolved in water and injected subcutaneously in a volume of 0.1–0.2 ml. All mice survived these treatments in excellent condition.

Receptor Binding Assay and Quantitative Autoradiographic Procedures

We used an *ex vivo* receptor binding assay largely as described [26]. In this assay, subjects are first treated *in vivo* with a receptor ligand and the degree of receptor occupancy is then assessed *in vitro*.

Mice were sacrificed by cervical dislocation. The cerebral hemispheres were rapidly removed, frozen in liquid nitrogen and stored at −70°C. A day later the hemispheres were homogenized in 20 volumes of ice-cold 50 mM Tris buffer (pH 8.0 at 27°C), containing 120 mM NaCl and 5 mM KCl, with 10 strokes of a motor-driven glass-Teflon homogenizer. The crude membranes were then immediately used without washing. Two hundred μl of the membrane preparation were incubated at 27°C with 1 nM ^{3}H-dihydroalprenolol (DHA; New England Nuclear; 90 Ci/m Mol) in a final volume of 500 μl of assay buffer. Samples were filtered under reduced pressure with Whatman GF/B filters that were then washed 5 times with 4 ml of ice-cold assay buffer. Specific binding, defined as the amount of DHA bound in the absence of competing ligand minus the amount bound in the presence of 1 μM (−)-propranolol, was 65–85% of total binding depending upon treatment of the mice. Assays were made in duplicate or triplicate.

An estimate was made of the loss of propranolol bound to receptors (following treatment with 50 μg/kg) that occurred during the homogenization and incubation periods. Dissociation of propranolol from receptors during homogenization was estimated by measuring the gain in DHA binding of an aliquot of a sample treated with 20 strokes over that of a second aliquot treated with 10 strokes of the homogenizer. Loss during incubation was estimated by measuring the increase in DHA binding of an aliquot of an homogenate that was pre-incubated for 30 min before adding DHA over that of an aliquot to which DHA was added at the start of the routine incubation period of 30 min. Total gain of specifically bound DHA estimated in these ways was 14% of the measured value, of which 4% occurred during homogenization (n=4), and 10% occurred during incubation (n=4). Assay values of DHA binding were correspondingly corrected.

The procedure [21] for quantitative autoradiography involved labelling 32 μ thick frozen sections of mouse brain *in vitro* with 300 pmol ^{125}I-pindolol (1P:2200 Ci/mmol), a non-selective beta antagonist. Sections of forebrain, diencephalon and brain stem were incubated with the ligand for 30 min at 23°, with a 10% loss of propranolol, as determined by pre-incubation and quantitative autoradiography. The sections were washed 20 min each with 3 changes of 4° buffer, dried on a 60° slide warmer, and applied against LKB Ultrofilm for 24 hr to generate autoradiograms. Optical densities in triplicate of selected areas of the autoradiograms were converted by computer into fm ligand/mg protein, using ^{125}I-brain-mash standards. Nonspecific binding was determined in the presence of 100 μM isoproterenol. In preliminary experiments, Scatchard analysis of density readings from the caudate-putamen indicated that the binding of IP was saturable, specific and of high-affinity (B max=400 f moles/g protein, Kd=60–90 pmol).

Other Procedures

Statistical analyses were made with the *t*-test.

The induction of amnesia by bitemporal injections of puromycin was taken to mean that engram spread had not occurred; the absence of amnesia following this treatment was taken to mean that the widespread engram was present.

TABLE 1

BEHAVORAL EXPERIMENTS TO TEST EFFECT OF (−)- AND (+)-PROPRANOLOL ON ENGRAM SPEED

Groups	Procedures	Initial Training Errors to Criterion	Relearning* % of Savings Errors	Engram spread
1.	(−)-Propanolol: Dose responses			
	a. Train $\xrightarrow{2\text{ days}}$ Saline $\xrightarrow{10\text{ days}}$ Puro (5)	7.8 ± 0.5	89.1 ± 8.7	
	b. Train $\xrightarrow{1\text{ day}}$ Drug 6 μg/kg days 2–4 $\xrightarrow{10\text{ days}}$ Puro (4)	9.8 ± 0.6	95.3 ± 2.3	+
	c. Train $\xrightarrow{1\text{ day}}$ Drug 12 or 25 μg/kg days 2–4 $\xrightarrow{10\text{ days}}$ Puro (10)	7.4 ± 0.6	37.2 ± 12.9	±
	d. Train $\xrightarrow{1\text{ day}}$ Drug 50 μg/kg days 2–4 $\xrightarrow{10\text{ days}}$ Puro (10)	8.2 ± 0.7	0.0	−
	e. Train $\xrightarrow{2\text{ days}}$ Drug 50 μg/kg $\xrightarrow{10\text{ days}}$ Puro (6)	6.2 ± 0.9	0.0	−
2.	(−)-Propranolol: Controls with 50 μg/kg			
	a. Train (a) $\xrightarrow{1\text{ day}}$ Drug $\xrightarrow{1\text{ day}}$ Reverse Train (b) $\xrightarrow{7\text{ days}}$ Test (6)	a. 9.2 ± 0.7 b. 8.5 ± 0.6	b. 92.0 ± 3.8	
	b. Train $\xrightarrow{1\text{ day}}$ Drug $\xrightarrow{1\text{ day}}$ Puro (4)	6.4 ± 1.0	0.0	−
	c. Train $\xrightarrow{9\text{ days}}$ Drug $\xrightarrow{10\text{ days}}$ Puro (4)	8.8 ± 0.8	80.3 ± 4.7	+
	d. Train $\xrightarrow{9\text{ days}}$ Drug $\xrightarrow{30\text{ days}}$ Puro (5)	8.1 ± 0.8	88.6 ± 7.9	+
	e. Train $\xrightarrow{9\text{ days}}$ Drug $\xrightarrow{10\text{ days}}$ Puro T+V+F(4)	6.7 ± 0.7	0.0	−
3.	(−)-Propranolol: Long term effect of 50 μg/kg of drug			
	a. Train $\xrightarrow{2\text{ days}}$ Drug $\xrightarrow{15,\ 25,\ 35\text{ or }60\text{ days}}$ Puro (20)	7.6 ± 0.5	2.4 ± 1.6	−
	b. Train $\xrightarrow{2\text{ days}}$ Drug $\xrightarrow{90\text{ days}}$ Puro (10)	7.7 ± 0.6	65.3 ± 14.2	+
	c. Train $\xrightarrow{90\text{ days}}$ Test (6)	6.2 ± 0.4	77.5 ± 5.9	
4.	(−)-Propranolol: Effect of 50 μg/kg given before training			
	a. Drug $\xrightarrow{20\text{ or }60\text{ days}}$ Train $\xrightarrow{10\text{ days}}$ Puro (10)	7.6 ± 0.9	8.7 ± 6.8	−
	b. Drug $\xrightarrow{90\text{ days}}$ Train $\xrightarrow{10\text{ days}}$ Puro (7)	7.1 ± 0.6	74.8 ± 14.2	+
5.	(+)-Propranolol: Dose responses of drug			
	a. Train $\xrightarrow{2\text{ days}}$ Drug 1250 or 2500 μg/kg $\xrightarrow{10\text{ days}}$ Puro (9)	9.1 ± 0.5	81.4 ± 4.8	+
	b. Train $\xrightarrow{2\text{ days}}$ Drug 3750 μg/kg $\xrightarrow{10\text{ days}}$ Puro (4)	6.5 ± 1.0	31.8 ± 18.6	±
	c. Train $\xrightarrow{2\text{ days}}$ Drug 5000 μg/kg $\xrightarrow{10\text{ days}}$ Puro (6)	5.8 ± 0.9	0.0	−

Time between procedures indicated over arrows. Puro=puromycin 2HCl neutralized with NaOH and except in Group 2e injected bitemporally. T+V+F=bitemporal + biventricular + bifrontal injections (10). Relearning tests 8–10 days after puro. Propranolol injected SC. Savings in Groups 1c, 3a, 4a and 5a were not significantly different at the multiple doses or times indicated. Number of mice per group in parenthesis. Negative savings scored as zero. Training and relearning results expressed as means ± SEM.

*In mice with bitemporal puro when relearning savings >65% engram spread considered consistently present; with low savings, inconsistently present or absent.

Behavioral-Experiment 1

After preliminary experiments with racemic propranolol, (−)-propranolol was used in experiments to establish a minimal subcutaneous dose. Following the dose schedule that produced positive results with inhibitors of dopamine β-hydroxylase [6], (−)-propranolol was injected for 3 consecutive days beginning on the day after training. Since the development of complete insensitivity to bitemporal puromycin normally requires about 6 days after training, puromycin was administered 10 days after the propranolol administration. Thus, if propranolol did not interfere with the normal maturation of memory, the long interval between training and bitemporal puromycin should insure that recall would be unaffected by puromycin.

Result and Discussion

As shown in Table 1 (group 1a and b), bitemporal puromycin failed to induce amnesia after treatment with saline or with 6 μg/kg of (−)-propranolol on days 2–4 after training. Increasing the propranolol dose to 12 or 25 μg/kg (group 1c) led to a significant reduction in group relearning savings ($p<0.01$) but the induction of amnesia was inconsistent. Treatment over the 3 day period with 50 μg/kg of propranolol consistently led to induction of amnesia by bitemporal injections of puromycin (group 1d). Finally, a single propranolol dose of 50 μg/kg, given 2 days after training, was found to be sufficient for consistent induction of amnesia by puromycin (group 1e).

Since a one-way analysis of variance showed that the errors to criterion during initial training were not statistically associated with the relearning scores, it appears that drug treatment was responsible for group differences in savings. We interpreted our findings to indicate that a single dose of 50 μg/kg of (−)-propranolol given 2 days after training consistently suppresses engram spread. This procedure was consequently used in all subsequent experiments. Mice treated in this way were normal in appearance and in cage

and maze behavior. Treatment of these mice with puromycin gave the typical symptoms of otherwise untreated mice—about 2 days of lethargy with reduced intake of food and water.

Behavioral Experiment 2

We next planned experiments, all with a single dose of 50 μg/kg of (−)-propranolol, to answer the following questions. (1) Does the drug affect learning or relearning in our mice? (2) Does it modify the amnesic effects of bitemporal or the 6 widespread injections of puromycin? Most importantly, might (−)-propranolol interact pharmacologically with the central nervous system in such a way as to make it more sensitive to the amnesic effect of puromycin so that bitemporal injections would be as effective as the 6 widespread injections normally required to induce amnesia 6 or more days after learning?

Results and Discussion

The effect of (−)-propranolol on learning and relearning was tested in a reversal experiment (Group 2a). Mice were treated with (−)-propranolol the day after they were trained to one arm of the Y-maze. One day after the drug treatment they were reverse trained to the opposite arm and 7 days later tested for relearning. As in past experiments with untreated mice [10], in those treated with (−)-propranolol there was no significant difference between the number of errors to criterion on first learning and those on reversal learning. Moreover, these mice had high relearning savings of their reversal training, i.e., there was no evidence that (−)-propranolol affected either learning or relearning of the Y-maze.

Nor did the drug modify the amnesic effects of puromycin. Consistent with its normal effect, puromycin bitemporally injected 2 days after training (1 day after (−)-propranolol) induced profound amnesia (group 2b). In groups 2c, d and e propranolol administration was delayed until 9 days after training to allow ample time for the development of insensitivity to bitemporal puromycin. Again consistent with their normal lack of effect, this treatment with puromycin failed to induce amnesia when given either 10 days (group 2c) or 30 days (group 2d) after propranolol. Profound amnesia did, as is normal, follow the 6 widespread injections of puromycin (group 2e).

These findings are consistent with our hypothesis that the effective engram is limited to the hippocampal area for 3 days after training and hence is vulnerable to bitemporal injections of puromycin whereas at later times the effective engram has a widespread distribution that requires widespread injections of puromycin to induce amnesia.

Behavioral Experiment 3

In Experiment 1, we found that post-training administration of (−)-propranolol (50 μg/kg) prolongs the normal period during which bitemporal injections of puromycin are amnesic. The following experiments were directed to determining the length of time necessary for these injections of puromycin, following post- or pre-training (−)-propranolol, to fail to induce amnesia.

All mice treated with (−)-propranolol received 50 μg/kg of the drug. When the drug was given 2 days after training, puromycin followed with an interval that varied from 15 to 90 days. When the drug was given before training, the interval between the 2 procedures varied from 20 to 90 days and puromycin followed 10 days after training to allow ample time for the normal development of insensitivity to bitemporal injections.

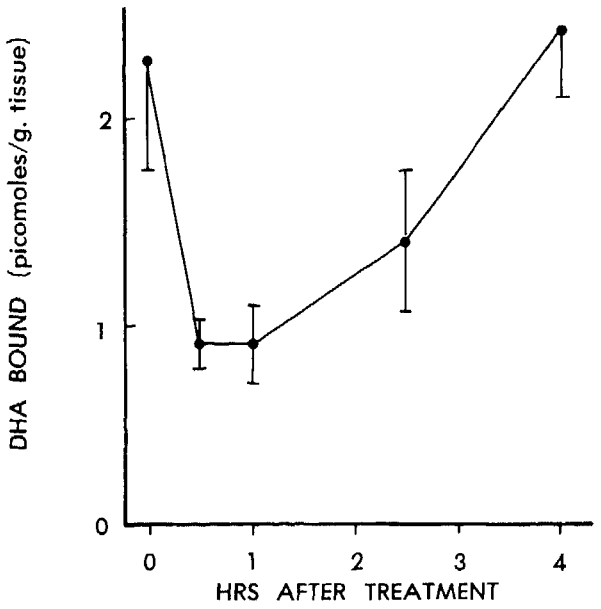

FIG. 1. Effect of a single subcutaneous dose of (−)-propranolol (50 μg/kg) on specific DHA binding of membranes prepared from the cerebral hemispheres. Mean±S.D. values are expressed as pmol DHA specifically bound per g of tissue. For the control and experimental groups, n=12 and 3–4, respectively.

Results and Discussion

Group 3a of Table 1 shows that bitemporal injections of puromycin produced profound amnesia 15, 25, 35 or 60 days after post-training treatment with (−)-propranolol. At 90 days, however, puromycin was ineffective (group 3b); the relearning savings of errors of this group were not significantly different from those of an untreated group (group 3c) that was tested for retention 90 days after training.

Much the same results were obtained when treatment with (−)-propranolol preceded training. In mice trained 20 or 60 days after treatment, bitemporally injected puromycin was amnesic (group 4a); extension of this interval to 90 days led to insensitivity to the puromycin injections (group 4b). The relearning savings of this last group were not significantly different from those of group 3b in which mice were treated with puromycin 90 days after post-training treatment with propranolol.

We interpret these findings to indicate that a single treatment with (−)-propranolol, either after or before training, suppresses the spread of the effective engram from temporal areas to widespread areas of the forebrain for 60–90 days as was found with repeated treatments using an inhibitor of dopamine β-hydroxylase [6]. During this interval, as was true with the inhibitor, engram spread occurred spontaneously.

Behavioral Experiment 4

To test the stereospecificity of (−)-propranolol's effect on puromycin-induced amnesia, the potency of the (+)-isomer

TABLE 2
OPITCAL DENSITY MEASUREMENTS OF BETA RECEPTORS

Structure	Specific IP binding at indicated times after treatment		
	Controls (n=2)	0.5 hr (n=1)	4 hr (n=2)
Layers 1 and 2 of:			
Parietal cortex	127.4	36.5 (29)	118.7 (93)
Occipital cortex	128.6	30.6 (24)	106.8 (83)
Entorhinal cortex	108.5	30.6 (28)	74.4 (69)
Caudate	128.4	37.6 (29)	124.0 (97)
Lateral septum	70.7	20.5 (29)	59.3 (84)
Medial preoptic area	58.5	20.1 (34)	80.4 (137)
Lateral posterior nu	99.1	18.4 (19)	83.1 (84)
Hippocampus CA1	94.5	21.7 (23)	76.0 (80)
Hippocampus CA3	65.1	11.8 (18)	46.1 (71)
Dentate gyrus	79.4	23.2 (29)	71.2 (90)
Reticular formation	46.4	9.3 (20)	40.8 (88)
Substantia nigra	97.5	31.7 (33)	58.6 (60)

Mice injected SC with 50 μg/kg of (−)-propranolol. Values are fmoles/mg protein. Values in parenthesis=% control value. IP=^{125}I-pindolol.

was examined (group 5 of Table 1). As in the experiment with (−)-propranolol (group 1e), (+)-propranolol was injected 2 days after training and was followed 10 days later by puromycin.

Results and Discussion

Bitemporally injected puromycin failed to induce amnesia after treatment with 1250 or 2500 μg/kg of (+)-propranolol (group 5a). Treatment with 3750 μg/kg (group 5b) caused an inconsistent but statistically significant loss of relearning savings ($p<0.05$) compared to the controls (group 1a) while 5000 μg/kg (group 5c) led to a consistent and profound amnesia.

We interpret these findings to indicate that (−)-propranolol was about 100 times as potent as (+)-propranolol in blocking development of the widespread engram.

Receptor Assay

The assays of beta-adrenergic receptors were made to test the relationship between the degree and duration of receptor blockade in the cerebral hemispheres caused by 50 μg/kg of (−)-propranolol and the drug's suppression of engram spread. As shown in Fig. 1, assays were made 0.5, 1, 2.5, and 4 hr after a single subcutaneous injection of 50 μg/kg of (−)-propranolol. As judged by the degree of binding of 1 nM DHA, 60% of the beta-receptors were blocked from 0.5 to 1 hr after treatment; full recovery from the antagonistic effects of (−)-propranolol appeared to have occurred 4 hr after treatment.

Quantitative Autoradiography

Quantitative autoradiography was used on a few samples to measure the constancy of the response of several regions of brain and brain stem to the subcutaneous injection of 50 μg/kg of (−)-propranolol (Table 2). Qualitatively, the results obtained with IP agreed with those obtained by the receptor assays mentioned above; there was a marked decrease in the specific binding of IP in all areas 0.5 hr after treatment followed by a large recovery of specific binding 3.5 hr later. In view of the variation observed in the studies with DHA, recovery may have been complete in all areas. Thus, as seen in Fig. 1, the S.D. of specific DHA binding of the untreated hemispheres was 25% of the mean.

DISCUSSION

Our observations lead to the following conclusions: (1) The suppression of engram spread by propranolol was stereospecific. (2) A single treatment with (−)-propranolol given either before or 2 days after training suppressed engram spread for 60–90 days. (3) In contrast, normal values of cerebral beta receptors (as judged by specific binding of DHA and IP) were reestablished approximately 4 hr after treatment with (−)-propranolol. The prolonged period of suppression of engram spread produced by propranolol following a short period of receptor blockade is similar to the prolonged period of suppression of engram spread produced by inhibitors of dopamine beta-hydroxylase following a short period of inhibition of norepinephrine synthesis [6]. (4) (−)-Propranolol, as we have used it, had no effect on learning or, in the absence of puromycin, no effect on relearning.

We are presently interested in exploring the role of the central adrenergic system in the development of an independent, effective engram from its initial locus in the temporal lobe to widespread cerebral areas. Our experiences with propranolol and with inhibitors of dopamine beta-hydroxylase suggest that the central adrenergic system is important in maintaining this aspect of mnemonic plasticity as it has been strongly suggested to be in maintaining the plasticity of the visual cortex [3,16].

There are, however, in our experiments, several ancillary effects of (−)-propranolol to be considered [24]. Two of these appear most important. First, there is the possibility that the drug's effect on the peripheral adrenergic system, following our subcutaneous injections, may account in whole

or in part for the suppression of engram spread [12]. Secondly, we cannot rule out the possibility that (−)-propranolol's interference with the serotoninergic system contributes to the suppression of engram spread. (−)-Propranolol is known to block serotonin (5HT, and $5HT_2$) receptors in addition to beta receptors [13,19], and also to inhibit 5HT uptake [18]. The possibility that 5HT receptors are importantly concerned is weakened by our finding that (+)-propranolol was about 100-fold weaker than the (−)-isomer in suppressing engram spread, whereas with 5HT, and $5HT_2$ binding sites of rat brains, the (+)-isomer has, respectively, only a 58- or 10-fold weaker affinity [13,19]. Using blood platelets as cellular models for investigation of membrane active drugs, racemic propranolol has been found to inhibit 5HT-uptake with an IC_{50} value of 4×10^{-6} M, and to produce 20% inhibition at a concentration of about 1×10^{-6} M [18]. Calcium uptake blockage [23], local anesthetic potency [1,28] and physical stabilization of membranes [17] appear to be of no significance in our experiments because the 2 isomers are equally effective in all these actions.

These considerations leave us with the problem of identifying the nature of those changes in the brain that follow both pre- and post-training treatment with (−)-propranolol and that account for the 60–90 day period necessary for the brain to recover its ability to initiate effective engram spread. A first step is to appraise the participation of temporal lobe structures in the development of the independent forebrain engram. Is the hippocampal area essential for this process? An affirmative answer appears to be provided by the experiments of Uretsky and McCleary [27]. They trained cats in a 1-way active-avoidance task. At 3 hr after training, the cats received a combined entorhinal-fornix lesion (hippocampal isolation) which resulted, on relearning 3–4 weeks after surgery, in a large, statistically significant ($p=0.002$) retention deficit compared to the unoperated controls. If, however, hippocampal isolation was delayed until 8 days after operation, and the cats were tested 3–4 weeks later there was no significant difference between their savings and those of the controls. Thus development of an independent forebrain engram required the presence of the hippocampal area for approximately 8 days after learning.

What may be the effect of (−)-propranolol on the postulated role of the hippocampal area? In their studies of labile and stable memory, Zornetzer *et al.* [31] have demonstrated in mice that a unilateral lesion of the locus coeruleus, made shortly after learning, dramatically extended the duration of labile memory during which electroconvulsive shock produces retrograde amnesia. Our results with (−)-propranolol may simply be another example of the role played by norepinephrine in the maturation of the memory trace; i.e., blockade of adrenergic receptors impairs the function of those neuronal programs which are essential for the formation of the independent forebrain engram. The prolonged period between recovery from receptor blockade and appearance of the forebrain engram suggests that these programs are highly dependent upon their microenvironment [15] and that reestablishment of the appropriate environment is a remarkably lengthy process.

ACKNOWLEDGEMENTS

We are grateful to Drs. Anders Hedberg and Howard Holtzer for rewarding discussions, to Dr. Barry Wolfe for the iodopindolol, and to the anonymous reviewers of this paper for their constructive suggestions. This work was supported in part by grant NS19597 from the National Institutes of Health to T.C.R. T.C.R. is a Fellow of the Esther A. and Joseph Klingenstein Fund and the Alfred P. Sloan Foundation.

REFERENCES

1. Barrett, A. M. and V. A. Cullum. The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br J Pharmacol* **34**: 43–55, 1968.
2. Brierly, J. B. Clinico-pathological correlations in amnesia. *Gerontol Clin* **3**: 97–109, 1961.
3. Daw, N. W., R. K. Rader, T. W. Robertson and M. Ariel. Effects of 6-hydroxydopamine on visual deprivation in the kitten striate cortex. *J Neurosci* **3**: 907–914, 1983.
4. Flexner, J. B. and L. B. FLexner. Restoration of expression of memory lost after treatment with puromycin. *Proc Natl Acad Sci USA* **57**: 1651–1654, 1967.
5. Flexner, L. B. and J. B. Flexner. Studies on memory: The long survival of peptidyl-puromycin in mouse brain. *Proc Natl Acad Sci USA* **60**: 923–927, 1968.
6. Flexner, J. B., L. B. Flexner and A. C. Church. Studies on memory: The cerebral spread of an engram in mice as affected by inhibitors of dopamine β-hydroxylase. *Pharmacol Biochem Behav* **18**: 519–523, 1983.
7. Flexner, L. B., J. B. Flexner, G. de la Haba and R. B. Roberts. Loss of memory as related to inhibition of protein synthesis. *J Neurochem* **12**: 535–541, 1965.
8. Flexner, L. B., J. B. Flexner and R. B. Roberts. Memory in mice analyzed with antibiotics. *Science* **155**: 1377–1383, 1967.
9. Flexner, L. B., J. B. Flexner, R. B. Roberts and G. de la Haba. Loss of recent memory in mice as related to regional inhibition of cerebral protein synthesis. *Proc Natl Acad Sci USA* **52**: 1165–1169, 1964.
10. Flexner, L. B., J. B. Flexner and E. Stellar. Memory in mice as affected by intracerebral puromycin. *Science* **141**: 57–59, 1963.
11. Flexner, L. B., P. Gambetti, J. B. Flexner and R. B. Roberts. Studies on memory: Distribution of peptidyl-puromycin in subcellular fractions of mouse brain. *Proc Natl Acad Sci USA* **68**: 26–28, 1971.
12. Gold, P. E., R. McCarty and D. B. Sternberg. Peripheral catecholamines and memory modulation. In: *Neuronal Plasticity and Memory Formation*, edited by C. A. Marsan and H. Matthies. New York: Raven Press, 1982.
13. Green, A. R., P. Johnson and V. L. Nimgaonkar. Interactions of β-adrenoceptor antagonists and agonists with the 5-hydroxytryptamine ($5\text{-}HT_2$) receptor. *Neuropharmacology* **22**: 657–660, 1983.
14. Haymaker, W., M. G. Smith, L. van Bogaert and C. de Chener. *Viral Encephalitis*. Springfield, IL: Charles C. Thomas Co., 1958, p. 95.
15. Holtzer, H. and J. Abbot. Oscillations of the chondrogenic phenotype in vitro. In: *The Stability of the Differentiated State*, edited by H. Ursprung. Heidelberg: Springer-Verlag, 1968.
16. Kasamatsu, T., J. D. Pettigrew and M. Ary. Recovery from effects of monocular deprivation. Acceleration with norepinephrine and suppression with 6-hydroxydopamine. *J Neurophysiol* **45**: 254–266, 1981.
17. Langslet, A. Membrane stabilization and cardiac effects of d,l-propranolol, d-propranolol and chlorpromazine. *Eur J Pharmacol* **13**: 6–14, 1970.
18. Lemmer, B., G. Wiethold, D. Hellenbrecht, I. J. Bak and H. Grobecker. Human blood platelets as cellular models for investigation of membrane active drugs: Beta-adrenergic blocking agents. *Naunyn Schmiedebergss Arch Pharmacol* **275**: 299–313, 1972.

19. Middlemiss, D. N., L. Blakeborough and S. R. Leather. Direct evidence for an interaction of beta-adrenergic blockers with the serotonin receptor. *Nature* **267**: 289–290, 1977.
20. Milner, B. *The Neurosciences: Third Study Program*. Cambridge, MA: MIT Press, 1974, pp. 75–89.
21. Rainbow, T. C., W. V. Bleisch, A. Biergon and B. S. McEwen. Quantitative densitometry of neurotransmitter receptors. *J Neurosci Methods* **5**: 127–138, 1982.
22. Rosenbaum, M., H. D. Cohen and S. H. Barondes. Effect of intracerebral saline on amnesia produced by inhibitors of cerebral protein synthesis. *Commun Behav Biol* **2**: 47–50, 1968.
23. Scales, B. and D. A. McIntosh. Effect of propranolol and its optical isomers on the radiocalcium uptake and the adenosine triphosphatase of skeletal and cardiac sarcoplasmic reticulum fractions. *J Pharmacol Exp Ther* **160**: 261–267, 1968.
24. Smith, H. J. The need to redefine membrane stabilizing activity of beta-adrenergic receptor antagonists. *J Mol Cell Cardiol* **14**: 495–500, 1982.
25. Squire, L. R. The neuropsychology of human memory. *Annu Rev Neurosci* **5**: 241–273, 1982.
26. Sriwatanakul, K. and S. R. Nahorski. Disposition and activity of B-adrenoceptor antagonists in the rat using an ex vivo receptor binding assay. *Eur J Pharmacol* **66**: 169–178, 1980.
27. Uretsky, E. and R. A. McCleary. Effect of hippocampal isolation on retention. *J Comp Physiol Psychol* **68**: 1–8, 1969.
28. Vaughan-Williams, E. M. Mode of action of beta-receptor antagonists on cardiac muscle. *Ann J Cardiol* **18**: 399–405, 1966.
29. Victor, M., J. B. Angevine, Jr., E. L. Maucall and C. M. Fisher. Memory loss with lesions of the hippocampal formation. *Arch Neurol* **5**: 244–263, 1961.
30. Woods, B. T., W. Schoene and L. Kneisely. Are hippocampal lesions sufficient to cause lasting amnesia? *J Neurol Neurosurg Psychiatry* **45**: 243–247, 1982.
31. Zornetzer, S. F., W. C. Abraham and R. Appleton. Locus coeruleus and labile memory. *Pharmacol Biochem Behav* **9**: 227–234, 1978.