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# Delayed Arousal From Anesthesia: A Further Similarity Between Stress and Beta-1 Adrenoceptor Blockade

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STONE, E. A., J. S. MANAVALAN AND D. QUARTERMAIN. *Delayed arousal from anesthesia: A further similarity between stress and beta-1 adrenoceptor blockade.* PHARMACOL BIOCHEM BEHAV **55**(1) 131–133, 1996.—The present studies investigated the role of beta adrenergic receptors in mediating arousal from anesthesia and the effects of stress on this process. In support of previous findings by others, it was found that blockade of beta-1 and beta-2 receptors by propranolol delayed arousal from halothane anesthesia and that this effect was attributable to blockade of beta-1 receptors because it was duplicated by betaxolol but not by ICI 118,551. Restraint stress also produced a delay in arousal from both halothane and hexobarbital anesthesia. This effect, which was observed at 0.5 but not 24 h after the stress, could not be explained by a stress-induced alteration in the metabolism of the anesthetic, as no difference in brain concentration of hexobarbital was found between stressed and control mice. The parallel effects of beta-1 blockade and stress further supports the hypothesis that stress produces an impairment in function at either the beta-1 receptor or some process coupled to this receptor.

Stress Arousal Anesthesia Beta adrenoceptor Betaxolol ICI 118,551

STRESS is known to induce a number of alterations of brain noradrenergic processes that may underlie various behavioral changes (9–11). Recently, we found that drugs that block central beta-1 adrenergic receptors could mimic the effects of acute stress on a number of active behaviors in the mouse (12). This has suggested that acute stress produces a deficit in neurotransmission at either the beta-1 receptor or in some pathway coupled to it.

Beta receptors have been linked to arousal and alerting processes (2,3). If transmission at or subsequent to these receptors is reduced after stress, it would be predicted that this would cause a diminished arousal. A simplified measure of arousal consists of determining the duration of anesthesia in rodents. It has been found that various beta receptor blocking agents prolong anesthesia caused by barbiturate and chloral hydrate stress would also have this effect.

The predominant beta receptor in the rodent forebrain is the beta-1 (7). Somewhat surprisingly, a previous study has suggested that the beta receptor mediating arousal from anesthesia is the beta-2 (5). However, the latter study utilized relatively high doses of a beta-2 receptor blocker, which may have affected beta-1 receptor function. Therefore, in addition to testing the effects of stress, we also reexamined the role of beta receptor subtypes in arousal using more selective doses of a beta-1 and -2 blocker.

## METHOD

Male Swiss–Webster mice (25–35 g) were used. The animals were housed in groups of five and were habituated to the laboratory for 3–4 days prior to the experiment. The animals were maintained with ad lib water and food under a 12 L:12 D cycle (lights on 0700 h). All injections of beta blockers were given SC 0.5 h prior to anesthesia. Restraint stress was administered by enclosing the animal in a cylindrical tube 3.5 cm in diameter, 7.5 cm long, with air holes for a 1-h period. This stress produces struggling, urination, defecation, and an elevated plasma corticosterone level (Stone, Unpublished observations). The stress was administered so that it terminated 0.5 or 24 h prior to the induction of anesthesia. Anesthesia was induced by halothane or hexobarbital. Halothane was administered for an 8-min period by vaporization with oxygen in a small chamber (15 × 10 × 10 cm). The concentration of the

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TABLE 1

EFFECT OF BETA BLOCKERS AND STRESS ON AROUSAL FROM HALOTHANE ANESTHESIA

| Treatment | Sleep Time (min) | |
|---|---|---|
| Pooled control | 2.91 ± 0.13 (24) | |
| Propranolol, 20 mg/kg | 6.74 ± 0.31 (6) | $p < 0.0001$ |
| Betaxolol, 20 mg/kg | 6.75 ± 1.21 (6) | $p < 0.05$ |
| ICI 118,551, 4 mg/kg | 3.67 ± 0.45 (6) | NS |
| Restrain stress | | |
| 0.5 h | 5.79 ± 0.55 (6) | $p < 0.01$ |
| 24 h | 3.30 ± 0.25 (6) | NS |

Values are means and SEMs of number of mice in parentheses. All groups tested against their respective control groups. All control groups have been pooled for purpose of presentation.

gas was 2%. Hexobarbital, dissolved in dimethylsulfoxide, was administered at 50 mg/kg IP in a total volume of 0.2 ml. Sleep time for hexobarbital was measured from the recovery of the righting reflex. This measure could not be used with halothane because it was unreliable in that animals, after showing a righting reflex, were still observed to be ataxic for several minutes. Therefore, we chose to use a different measure for halothane recovery—the resumption of the rearing response—because this was only observed after the animals were completely recovered from the anesthetic.

Brain hexobarbital levels were assayed in the whole brains of independent groups of control and restrained mice. The animals were treated as above, with the stressor and hexobarbital (50 mg/kg). Ten minutes after injection the anesthetized animals' brains were removed, frozen, and subsequently homogenized in distilled water. An aliqout of the homogenate was buffered to pH 6.5 and extracted with methylene chloride. Following evaporation of the solvent, the residue was reconstituted with mobile phase and injected on a reversed-phase ODS column and eluted with a phosphate buffer/acetonitrile mobile phase. All samples were analyzed with a six-point calibration standard curve using alphenol as the internal standard.

Each drug or stress group was run with a corresponding control as a planned comparison and was evaluated statistically with an independent *t*-test.

## RESULTS

The experiments utilizing halothane are shown in Table 1. The nonselective beta antagonist, *dl*-propranolol at 20 mg/kg produced a significant 179% lengthening of the recovery period. The beta-1 selective antagonist, betaxolol, at 20 mg/kg produced a significant 113% increase. The beta-2 selective antagonist, ICI 118,551 was ineffective at 4 mg/kg. Restraint stress produced a significant 63% increase at 0.5 h but had no effect at 24 h prior to anesthesia.

The stress experiment was replicated using hexobarbital as the anesthetic (Table 2). Restraint stress was found to significantly increase hexobarbital sleeping time (97% increase). The stress had no effect on the concentration of hexobarbital in the brain at 10 min after injection (in μg/g: control, 28.6 ± 4.6; stressed, 28.9 ± 3.6, $n = 6$).

## DISCUSSION

The present studies investigated the effects of beta adrenergic receptor antagonists and stress on arousal from anesthesia.

TABLE 2

EFFECT OF STRESS ON HEXOBARBITAL SLEEPING TIME

| Treatment | Sleep Time (min) | |
|---|---|---|
| Control | 21.1 ± 3.4 (7) | |
| Restraint stress: 0.5 h | 36.6 ± 5.4 (7) | $p < 0.05$ |

Values are means and SEM of number of mice in parentheses.

It was found that nonselective blockade of beta-1 and beta-2 receptors with propranolol or selective blockade of beta-1 receptors with betaxolol lengthened the recovery time from halothane anesthesia but selective blockade of beta-2 receptors with ICI 118,551 did not. These findings are in agreement with previous research showing that nonselective blockade of beta receptors prolongs barbiturate and chloral hydrate anesthesia (1,4,5). They differ, however, from a previous report showing that beta-2 but not beta-1 blockade produces the effect (5). However, as discussed in the introduction, the latter study used relatively high doses of ICI 118,551, which may have blocked beta-1 receptors. Also, these authors used a beta-1 antagonist, metoprolol, which has not been studied for its ability to penetrate the rat's blood–brain barrier. The present study utilized drugs that are established to enter the rat brain, and employed doses of betaxolol and ICI 118,551 that have been shown in extensive dose response in vivo binding experiments to be selective for the corresponding receptor subtypes allowing for differences in metabolism between mice and rats (14). Therefore, it is concluded that beta-1 rather than beta-2 receptors mediate recovery from halothane anesthesia and presumably barbiturate anesthesia as well.

Restraint stress was found to significantly prolong halothane anesthesia. That this was not specific to halothane was shown by the finding that restraint also prolonged hexobarbital anesthesia. This effect was unlikely to be due to a stress-induced alteration in the metabolism of the anesthetic because assay of hexobarbital revealed no difference between stressed and nonstressed animals. It is concluded, therefore, that stress retards processes that arouse animals from anesthesia.

Previous studies have shown that stress and beta-1 receptor blockers have similar effects on locomotor activity and grooming. The present finding indicates that they also have similar actions on the duration of anesthesia to hexobarbital and halothane. Taken together, these findings strongly suggest that stress reduces transmission at beta-1 receptors or in some pathway coupled to these receptors, which results in a reduced poststress arousal.

The present findings also indicate that the effect of stress is relatively short lived. While it was present at 0.5 h, it was absent 24 h later. This suggests that the change responsible for this effect is a transient one. Transient biochemical changes that would impair the action of beta-1 receptors in the brain include receptor desensitization and the depletion of brain glycogen whose hydrolysis is partially under the control of forebrain beta-1 receptors (8). Stress is known to induce the desensitization of adrenergic receptors in the brain (13,15) and also to deplete central stores of glycogen (6). We have recently shown that pretreatment of animals with a beta-1 blocker in such a way that the drug is present during the stress and should retard receptor desensitization and glycogen depletion but is not present during the subsequent behavioral test has the effect of reversing certain behavioral actions of

the stress (Stone and Quartermain, in preparation). Further research on the role of beta receptor desensitization and glycogen depletion in the present phenomenon are, therefore, warranted.

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