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The Role of Serotonergic Pathways in Isolation-Induced Aggression in Mice[1]

JEFFREY B. MALICK[2] AND ALLEN BARNETT

Department of Pharmacology, Biological Research Division
Schering Corporation, Bloomfield, New Jersey 07003

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MALICK, J. B. AND A. BARNETT. *The role of serotonergic pathways in isolation-induced aggression in mice.* PHARMAC. BIOCHEM. BEHAV. 5(1) 55–61, 1976. – Male mice that became aggressive following four weeks of social isolation were treated with seven known serotonin receptor antagonists. All of the antiserotonergic drugs selectively antagonized the fighting behavior of the isolated mice; the antiaggressive activity was selective since, at antifighting doses, none of the drugs either significantly altered spontaneous motor activity or impaired inclined-screen performance. Antagonism of 5-HTP-induced head-twitch was used as an in vivo measure of antiserotonergic activity and a statistically significant correlation existed beteen potency as an antiserotonergic and potency as an antiaggressive. PCPA, a serotonin depletor, also significantly antagonized isolation-induced aggression for at least 24 hr postdrug administration. The interrelationship between cholinergic and serotonergic mechanisms in the mediation of isolation aggression was investigated. The involvement of serotonergic systems in isolation-induced aggression is discussed.

Isolation-induced aggression Physostigmine lethality Serotonergic pathways Spontaneous motor activity
Parachlorophenylalanine 5-HTP-induced head-twitch Serotonin receptor antagonists

PREVIOUS studies that attempted to determine the possible role of serotonergic mechanisms or indoleamine systems in aggressive behavior resulted in conflicting results. Serotonergic systems appeared to influence aggression, but their influence appeared to be quite different depending upon what model of aggression was investigated.

Parachlorophenylalanine (PCPA), a specific depletor of brain serotonin [25], has been shown to attenuate the irritability and aggressiveness of rats with septal lesions [12]. On the other hand, several investigators [4, 5, 30] have failed to significantly alter footshock-induced fighting behavior in rats following PCPA administration; Ellison and Bresler [14] recently reported increased shock-elicited aggression following PCPA.

The effects of PCPA on muricidal behavior in rats have been studied extensively. Several authors [10, 13, 34] have reported that PCPA converted non-killer rats to killers and McLain and Cole [30] reported increased attack frequencies and decreased latency to attack in killers following PCPA. Lesions of the median and dorsal raphe that resulted in 70% depletion of forebrain serotonin (5-HT) also resulted in the induction of mouse-killing [21]. It has also been repeatedly demonstrated [10, 28, 43] that 5-hydroxytryptophan will inhibit muricidal behavior. Thus, 5-HT appears to act as an inhibitory transmitter with regard to muricidal behavior.

Welch and Welch [44] reported that PCPA transiently abolished isolation-induced aggression in mice; however, this effect did not appear to be related to decreased brain serotonin. Biochemically, many investigators have studied serotonin brain levels and turnover in isolated mice. Most investigators [9, 19, 20, 31] found no significant differences in brain 5-HT levels in isolated versus control group-housed mice, except for one report of increased 5–HT levels [15]. When turnover of 5-HT was studied, no significant changes were observed in two studies [15,20] but in the majority of studies, decreased turnover rates were consistently observed in isolated mice [16, 38, 40, 44]. Recently, lesions of the raphe nuclei that signficantly reduced 5-HT levels in the forebrain have been shown to abolish isolation aggression in mice [26].

The present studies were designed to further investigate the role of serotonergic pathways in isolation-induced aggression in mice.

EXPERIMENT 1: EFFECTS OF SEROTONIN RECEPTOR ANTAGONISTS ON ISOLATION-INDUCED AGGRESSION

This study was designed to determine the effects of antiserotonergic drugs on isolation-induced aggression in mice. Previously, only one report mentions the activity of an antiserotonergic drug, methysergide, on aggressive behavior induced by isolation in mice; in this study methysergide (0.4 mg/kg, IP) failed to inhibit the aggressive behavior [41]. Seven drugs which have been reported to be serotonin receptor antagonists (i.e., drugs which inhibit the

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[2] Requests for reprints should be sent to Dr. Jeffrey B. Malick, Biomedical Research Department, ICI United States Inc., Wilmington, Delaware 19897.

TABLE 1

EFFECTS OF SEROTONIN RECEPTOR ANTAGONISTS ON ISOLATION-INDUCED AGGRESSION IN MICE

Treatment	Antagonism of isolation-induced fighting ED50 (mg/kg, i.p.) (95% fiducial limits)*	N†	Impairment of inclined screen performance in isolated mice NTD50 (mg/kg, i.p.) (95% fiducial limits)*	N‡
Methiothepin	0.04 (0.02-0.09)	70	0.8 (0.6-1.1)	140
Mianserin	0.5 (0.2-1.3)	45	>20	90
Methysergide	1.0 (0.6-1.4)	44	>20	88
Cyproheptadine	1.1 (0.4-2.1)	35	>20	70
Pizotyline	1.5 (0.9-2.3)	59	>25	118
Xylamidine	2.5 (1.1-5.0)	55	>20	110
Cinanserin	7.3 (5.3-12.1)	147	>25	294

*ED50's and 95% fiducial limits were calculated by Probit Maximum Likelihood Analysis [17].
†Number of pairs of mice used for each drug.
‡Number of mice used for each drug.

pharmacological effects of serotonin at specific receptor sites) were used in this study: cinanserin [18]; cyproheptadine [36]; methiothepin [32]; methysergide [11]; mianserin [42]; pizotyline [27]; and xylamidine [7].

Method

Isolation-induced aggression in mice was produced by a modification of the method of Yen and co-workers [45] and has been reported previously [1]. Briefly, CF No. 1-S male mice (18–22 g) were isolated for a period of 4 weeks and then tested for aggression by placing an isolated mouse into the home cage of another isolate. Pairs of mice were observed for 3 min, and presence or absence of fighting was recorded. All pairs were tested for aggression on the day before drug administration and only those which were consistent fighters were used for drug studies. Mice were retested for aggression 30 min after drug administration. Immediately following the aggression test, the mice were checked for neurological impairment by gently placing them on a 45° inclined screen; any mouse that exhibited impaired performance was scored as being ataxic. Established fighters were re-used for drug studies allowing one week between studies.

All drug doses were calculated in terms of mg/kg of free base and were either dissolved in distilled water or suspended in an 0.4% methylcellulose vehicle. All drugs were administered via the intraperitoneal (IP) route in a volume of 10 ml/kg. The drugs used in this study were the maleate salts of methiothepin, methysergide and pizotyline, the hydrochloride salts of cinanserin, cyproheptadine and mianserin, and xylamidine tolsulfate.

The ED50, that dose which prevented fighting in 50% of the pairs, and the 95% fiducial limits were calculated for each drug by Probit Maximum Likelihood Analysis [17]. The NTD50, that dose which caused impairment of inclined screen performance in 50% of the mice tested, was calculated as above wherever possible.

Results and Discussion

Table 1 summarizes the results of this experiment. All seven of the serotonin receptor antagonists that were tested antagonized fighting in isolated mice. With each drug, the antagonism of aggression was selective in that the mice did not exhibit concurrent neurological impairment on the inclined screen at doses which inhibited fighting (Table 1). Methiothepin was the only drug which produced significant ataxia at doses below 20–25 mg/kg, IP. Although methiothipin produced neurological impairment at relatively low doses, its therapeutic ratio (NTD50/ED50) was 20, which indicates that it was still a very selective antiaggressive effect.

EXPERIMENT 2: EFFECTS OF ANTISEROTONERGIC DRUGS ON SPONTANEOUS MOTOR ACTIVITY

Agents which alter spontaneous motor activity might affect aggressive behavior. It was therefore important to investigate whether the serotonin receptor antagonists would affect spontaneous motor activity at doses that inhibited aggression. Since isolation-induced aggressive mice sometimes exhibit differences in drug sensitivities when compared to group-housed mice [2, 6, 20, 39], all drugs were tested in both group-housed and isolated mice.

Method

Spontaneous motor activity of mice was measured utilizing photocell chambers (Woodard Manufacturing Corporation). Each chamber was circular, and the walls contained six photocells spaced equally about a center core. Each time a mouse broke a beam of light going to one of the photocells, one unit of motor activity was recorded automatically. Only one mouse was placed in each cham-

TABLE 2

EFFECT OF SEROTONIN RECEPTOR ANTAGONISTS ON SPONTANEOUS MOTOR ACTIVITY IN ISOLATED AND GROUP-HOUSED MICE

Treatment	Dose (mg/kg, i.p.)*	Spontaneous Motor Activity (% change from control)† Isolated mice‡	Group-housed mice‡
Methiothepin	0.1	−22.6	− 5.7
Mianserin	1.0	− 9.7	−22.6
Methysergide	2.0	−15.9	−14.1
Cyproheptadine	2.2	+34.8	−21.8
Pizotyline	3.0	−33.7	−21.2
Xylamidine	5.0	+ 4.2	− 7.0
Cinanserin	14.6	−33.7	−32.6

*Dose = approximately 2 x ED50 for antagonism of isolation-induced fighting
†Drug-treated group compared to saline-treated control group by ANOVA.
‡Five mice per group were used for all treatments.

ber, and the chambers were placed within a darkened, sound-attenuated box. Drugs were administered IP at doses approximately 2 times their respective ED50's for antagonism of isolation-induced fighting; these doses were chosen to make sure that nonspecific affects were not responsible for the antiaggressive activity even at the upper end of the dose-response curves. Mice were placed in the chambers 30 min after drug administration, and motor activity was recorded for the next 30 min. Results were expressed as total counts per 30 min test session and were analyzed statistically using analysis of variance, comparing drug-treated groups to appropriate saline-treated controls.

Results and Discussion

The results of this experiment are summarized in Table 2. The data are expressed as percent change in spontaneous motor activity compared to the appropriately treated saline control groups. At the doses tested, which were twice the antiaggressive ED50 doses, none of the serotonin receptor antagonists significantly ($p>0.05$; ANOVA) altered spontaneous locomotor activity in either group-housed or isolated mice.

Since none of the serotonin receptor antagonists either impaired inclined screen performance (Experiment 1; see Table 1) or caused significant decreases in locomotor activity even at twice their antifighting ED50 doses, it was concluded that they were selective antagonists of this form of aggression in mice.

EXPERIMENT 3: RELATIONSHIP BETWEEN IN VIVO ANTISEROTONERGIC ACTIVITY AND ANTIFIGHTING ACTIVITY

The purpose of this experiment was to determine whether a correlation existed between potency as an antiaggressive and potency as an antiserotonergic for the antagonists tested in Experiment 1. Antagonism of 5-hydroxytryptophan (5-HTP)-induced head-twitch [8] was used as an in vivo measure of serotonin antagonism in the central nervous system (CNS) of the mouse. This procedure is reported to be an in vivo measure of serotonin receptor antagonism in the CNS since the head-twitch induced by 5-HTP in mice is apparently due to a specific action of serotonin on neurons in the brainstem because of the following observations: (1) it is produced after the intraperitoneal injection of 5-HTP but not following parenteral serotonin administration [8], and it has been shown that 5-HTP crosses the blood-brain barrier whereas serotonin does not [37]; (2) it is antagonized by the administration of decarboxylase inhibitors which prevent the conversion of 5-HTP to serotonin [8]; (3) it is potentiated by monoamine oxidase inhibitors which prevent the breakdown of newly formed serotonin [8]; and, (4) the time-course of the elevation of serotonin levels in the brainstem following 5-HTP administration correspond to the time-course of the head-twitch response, whereas whole brain serotonin levels do not [8].

Since at least one of the well-known indoleamine antagonists, cyproheptadine [36], had previously been shown to possess potent anticholinergic activity [1], and anticholinergic drugs are selective antagonists of isolation-induced aggression in mice [1, 9, 22], the serotonin receptor antagonists were also tested for anticholinergic activity. Inhibition of physostigmine lethality, which has been shown to be a measure of central anticholinergic activity [29], was used as an in vivo measure of this activity in mice.

Method

Antagonism of 5-HTP-induced head-twitch. 5-Hydroxytryptophan (5-HTP) produces head-twitching (distinctive side to side flicking of the head independent of body jerking) in mice. Pretreatment with pargyline HCl, a monoamine oxidase inhibitor, was used to prevent the metabolism of the serotonin (5-HT) resulting from the conversion of 5-HTP, thus potentiating the head-twitch response. Five male mice were used to evaluate each dose of the serotonin receptor antagonists. Pargyline (100 mg/kg, IP) was given 2½ hr prior to the intraperitoneal administration of test drug; 30 min after test drug treatment, the mice were challenged with 5-HTP (25 mg/kg, IP). Head-twitches were counted between 10 and 20 min post 5-HTP administration. The mean number of head-twitches per mouse was determined for each test group, and this value was compared to the same day control value. The dose intervals for each test drug were kept constant (0.5 $\log_{10}$ units). The MED50, the minimal dose of the test drug that produced a

TABLE 3

INHIBITION OF ISOLATION-INDUCED AGGRESSION, 5-HTP-INDUCED HEAD-TWITCH AND PHYSOSTIGMINE-INDUCED LETHALITY IN MICE BY SEROTONIN ANTAGONISTS

Treatment	ED50 (mg/kg, i.p.) for antagonism of isolation-induced fighting (95% fiducial limits)*	Antagonism of 5-HTP-induced head-twitch MED50 (mg/kg, i.p.)†	N‡	ED50 for antagonism of physostigmine-induced lethality (mg/kg, i.p.) (95% fiducial limits)*	N‡
Methiothepin	0.04 (0.02-0.09)	0.3	20	>20	40
Mianserin	0.5 (0.2-1.3)	0.3	20	>40	20
Methysergide	1.0 (0.6-1.4)	0.3	145	>80	40
Cyproheptadine	1.1 (0.4-2.1)	0.3	50	0.7 (0.3-1.0)	30
Pizotyline	1.5 (0.9-2.3)	0.3	60	1.9 (1.2-2.9)	54
Xylamidine	2.5 (1.1-5.0)	3.0	65	3.4 (0.1-3.5)	61
Cinanserin	7.3 (5.3-12.1)	10.0	65	>80	130

*ED50's and 95% fiducial limits were calculated by Probit Maximum Likelihood Analysis [17].
†Minimal effective dose for producing 50% or greater reduction of head-twitch.
‡Number of mice used for each drug.

50% or greater reduction in head-twitches as compared to the saline control group for that day, was calculated for each of the antiserotonergic drugs tested. The dose-response curves generated in this procedure did not permit ED50 determination via Probit Maximum Likelihood Analysis because the slopes of the curves were too steep.

Inhibition of physostigmine-induced lethality in mice. The method used was a modification of the technique reported by Collier and co-workers [3]. Physostigmine salicylate (1.0 mg/kg, subcutaneously) produced 100% lethality when administered to mice grouped 10 per cage (5 x 11 x 5 in.) 20 min after drug administration. Test agents were administered intraperitoneally 30 min prior to the physostigmine. Inhibition of lethality was then calculated as percent of control. The ED50, that dose which prevented lethality in 50% of the mice, was calculated for each drug by Probit Maximum Likelihood Analysis [17].

Results and Discussion

The results of these studies are summarized in Table 3. The order of listing of the serotonin receptor antagonists was according to their order of potency as antagonists of isolation aggression, and their respective antifighting ED50 values were listed for comparative purposes. Antiserotonergic activity was significantly correlated with antifighting potency (r_s = 0.802; $p<0.05$; Spearman rank correlation coefficient [35]).

When the indoleamine antagonists were tested for their ability to inhibit physostigmine lethality, three of them (cyproheptadine, pizotyline, and xylamidine) were also found to possess significant anticholinergic activity (Table 3). The other serotonin receptor antagonists, methiothepin, mianserin, methysergide, and cinanserin, were completely devoid of anticholinergic activity even at doses that were many times their antiserotonergic or antifighting dose ranges (Table 3).

Therefore, at least four of the antiserotonergic drugs (i.e., methiothepin, mianserin, methysergide, and cinanserin) appeared to antagonize aggression selectively due to their action at serotonin receptors. The antiaggressive properties of the other three drugs (i.e., cyproheptadine, pizotyline, and xylamidine) may have been due to the interaction or combined effects of their antiserotonergic and anticholinergic activities. In addition, since there was a significant correlation between potency as a serotonin receptor blocker and potency as an antiaggressive when both the specific and nonspecific (i.e., cyproheptadine, pizotyline, and xylamidine) blockers were compared, the antiserotonergic activity may be the predominant activity responsible for the antifighting activity in isolated mice with all of these drugs.

EXPERIMENT 4: INTERACTION BETWEEN ANTISEROTONERGIC AND ANTICHOLINERGIC DRUGS ON AGGRESSION

Since three of the antiserotonergic drugs were also found to possess anticholinergic activity, we decided to study the combined pharmacological effects of these two classes of drugs. Scopolamine hydrobromide was used in these studies because it was a potent antagonist of isolation aggression (ED50 = 1.3 (0.8–1.9) mg/kg, IP) and also a potent inhibitor of physostigmine lethality (ED50 = 0.02 (0.01–0.04) mg/kg, IP); scopolamine was devoid of antiserotonergic activity at 10 mg/kg, IP, a dose more than 500 times its ED50 for anticholinergic activity. Methysergide was used as the antiserotonergic in these studies because it was devoid of anticholinergic activity at a dose greater than 250 times its MED50 for antagonism of 5-HTP head-twitch (Table 3).

Method

Relatively low doses (less than ED50's from previous experiments) of methysergide and scopolamine were given

alone and in combination to separate groups of isolated mice (5 pairs/dose) to determine whether drug interactions (e.g., summation, antagonism or potentiation) would be observed.

Results and Discussion

The results of the interaction studies are shown in Fig. 1. No potentiation or additive antiaggressive effects were observed at any of the doses of methysergide and scopolamine. Thus, as a result of this preliminary study, both cholinergic and serotonergic systems appear to be involved in the control of isolation aggression in mice; however, the two systems may possibly act independently of one another since their respective receptor antagonists apparently did not interact pharmacologically in this study.

EXPERIMENT 5: EFFECTS OF SEROTONIN SYNTHESIS INHIBITION ON ISOLATION-INDUCED AGGRESSION

PCPA has been shown to be a relatively selective depletor of brain serotonin in mice [25]; it inhibits 5-HT biosynthesis by inhibition of the rate-limiting enzyme, tryptophan hydroxylase [23]. Welch and Welch [44] completely abolished isolation aggression at 10 min post PCPA (360 mg/kg, IP); however, they observed a gradual diminution of the response with time such that only 25% of the mice were inhibited 5 hr postdrug. This effect didn't appear to be correlated with changes in brain 5-HT levels since at 10 min post PCPA they observed slight but signficant decreases in norepinephrine but no changes in 5-HT or dopamine; only small but significant decreases in 5-HT were observed at 3 and 6 hr post PCPA.

Kilian and Frey [24] observed that brain levels of serotonin were significantly reduced (58.1% decrease) in mice 24 hr following a high dose (900 mg/kg, PO) of PCPA whereas no significant alterations were observed in the brain levels of the catecholamines (norepinephrine and dopamine). The present experiment was designed to look at both the immediate (30 min postdrug) and the long-term (24 and 48 hr) effects of PCPA on isolation aggression.

Method

The mice were handled and tested the same as in Experiment 1. The mice were administered various doses of PCPA IP and retested for aggression at 30 min and 24 hr postdrug. ED50's and 95% fiducial limits were calculated for both time intervals by Probit Maximum Likelihood Analysis [17].

The time course of the behavioral inhibition following PCPA was followed in a group of 10 pairs of mice that had received 1000 mg/kg, IP; these animals were retested at 30 min and 24 and 48 hr post PCPA.

Results and Discussion

The results of this study confirm the finding by Welch and Welch [44] that PCPA significantly inhibits fighting shortly after drug administration (Table 4); the ED50 for antagonism of fighting was 148 mg/kg, IP 30 min post PCPA administration. This dose-related rapid effect of PCPA which presumably occurs prior to significant depletion of 5-HT levels [44] may either be due to a direct action of PCPA or to short-lived changes in catecholamine balance in the brain. A dose-related antagonism of fighting was still present 24 hr after PCPA administration, although

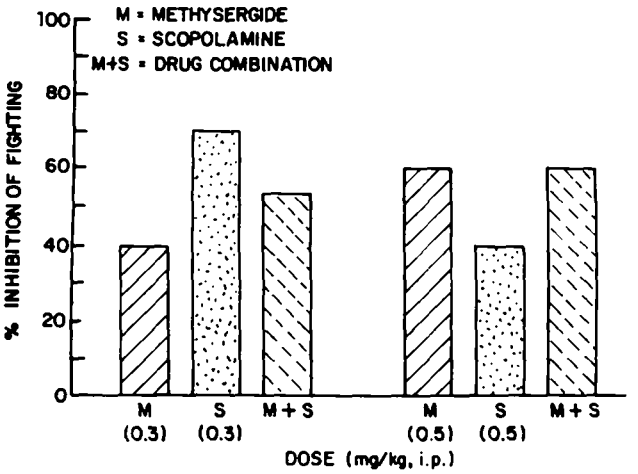

FIG. 1. Interaction between antiserotonergic and anticholinergic drugs in isolation-induced aggression in mice.

the magnitude of the effect (ED50 = 403 mg/kg, IP) was significantly less than was observed at 30 min postdrug (Table 4). This observation is in contrast to previous results [44] in which the mice were no longer significantly affected 5 hours post PCPA. PCPA did not impair inclined screen performance at any of the doses or time intervals studied. The results of this study indicated that significant inhibition of fighting occurred at a time (24 hr) at which PCPA has been reported to produce selective (i.e., no significant changes in catecholamines were observed) depletion of brain 5-HT in isolated [44] and group-housed [24] mice.

The duration of the antiaggressive effects of PCPA appeared to be between greater than 24 hr but less than 48 hr since highly significant inhibition of aggression was observed at 30 min and 24 hr postdrug (1000 mg/kg, IP) but returned to within control levels by 48 hr postdrug (Table 4). In rats, PCPA causes a marked decrease in brain serotonin which lasts for several days [25]; however, in mice much larger doses of PCPA [24] are required to significantly deplete brain serotonin, and the depletion does not appear to last as long as following a single dose of PCPA in rats.

TABLE 4

INHIBITION OF ISOLATION AGGRESSION BY PCPA

Treatment	Dose (mg/kg, i.p.)	Inhibition of fighting (No. of pairs inhibited/No. of pairs tested) (Time after drug administration) 30 min	24 hr	48 hr
PCPA	100	1/4	—	
	150	5/10	1/10	
	300	9/10	5/10	
	600	5/5	3/5	
	1000	10/10	8/10	2/10
ED50 (mg/kg, i.p.) for inhibition of fighting (95% fiducial limits)*		148.4 (107.1-205.7)	403.1 (237.3-709.3)	—

*ED50 and 95% fiducial limits calculated by Probit Maximum Likelihood Analysis [17].

GENERAL DISCUSSION

The seven serotonin receptor antagonists studied were shown to be potent, selective antagonists of isolation-induced aggression in mice; the antiaggressive activity was selective since, at antifighting doses, none of the drugs either significantly altered spontaneous motor activity or impaired inclined-screen performance. Since the antiserotonergics were all adminstered systemically, there is the possibility that the antiaggressive effects were due to indirect actions at serotonin receptors in the periphery (e.g., thyroid, pancreas) which in turn caused alterations in hormonal levels, etc. that ultimately resulted in the behavioral modification. Since the effects occurred very rapidly (by 30 min postdrug) is is doubtful that secondary actions resulted in the inhibition of aggression. In addition, the antiaggressive activity of the antiserotonergics tested is believed to be due to their actions at serotonin receptor sites in the CNS since their potency as an antagonist of isolation-induced fighting was significantly correlated with their in vivo potency as antagonists of the 5-HTP-induced head-twitch response, which has been shown to be a measure of serotonin receptor blockade in the CNS [8].

In addition, PCPA, a depletor of serotonin in the brain of mice [25], selectively inhibited fighting at a time (24 hr postdrug) at which serotonin levels have been shown to be significantly lowered without concurrent significant changes in catecholamine levels [24, 25, 44]. The inhibition of aggression observed at 30 min post PCPA administration, which confirms the observation of Welch and Welch [44], cannot be accounted for in terms of serotonin depletion; rather, this immediate activity may be either due to some direct effect of PCPA itself or the result of an imbalance in biogenic amines. However, since inhibition of aggression occurred at a time (24 hr) following PCPA administration in which only serotonin is significantly depleted, serotonin depletion resulted in an inhibition of aggression in isolated mice.

As a result of this study, there now appears to be at least two neurochemical systems, one serotonergic and the other cholinergic [1, 9, 22] involved in the regulation of isolation-induced aggression in mice. The preliminary results of Experiment 4 appear to predict that the two systems may act independently of one another since the activities of scopolamine and methysergide were not additive; however, the interrelationship between these neurotransmitter systems in isolation aggression is not clearly understood at this time.

Previous investigations have shown that serotonin turnover is decreased in isolated, aggressive mice [16, 38, 40, 44] and that lesions of the raphe, which significantly decreased serotonin levels in the brain, inhibited this form of aggression [26]. In this study, it has been demonstrated that isolation-induced aggression in mice can be selectively antagonized by either serotonin receptor blockade or depletion of serotonin via synthesis inhibition. The results of these experiments support the conclusion that serotonergic pathways are significantly involved in the regulation of isolation-induced aggression in mice.

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