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Does Learned Helplessness Induction by Haloperidol Involve Serotonin Mediation?

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PETTY, F., G. KRAMER AND M. MOELLER. *Does learned helplessness induction by haloperidol involve serotonin mediation?* PHARMACOL BIOCHEM BEHAV **48**(3) 671–676, 1994.—Learned helplessness (LH) is a behavioral depression following inescapable stress. Helpless behavior was induced in naive rats by the dopamine D_2 receptor blocker haloperidol (HDL) in a dose-dependent manner, with the greatest effects seen at 20 mg/kg (IP). Rats were tested 24 h after injection. Haloperidol (IP) increased release of serotonin (5-HT) in medial prefrontal cortex (MPC) as measured by in vivo microdialysis. Perfusion of HDL through the probe in MPC caused increased cortical 5-HT release, as did perfusion of both dopamine and the dopamine agonist apomorphine. Our previous work found that increased 5-HT release in MPC correlates with the development of LH. The present work suggests that increased DA release in MPC, known to occur with both inescapable stress and with HDL, may play a necessary but not sufficient role in the development of LH. Also, this suggests that increased DA activity in MPC leads to increased 5-HT release in MPC and to subsequent behavioral depression.

Learned helplessness Dopamine Haloperidol Serotonin Medial prefrontal cortex Animal models Microdialysis

LEARNED helplessness is a behavioral deficit caused by inescapable stress and is proposed as an animal model for human depressive illness. Studies on the neurochemical basis of this behavior have long implicated dopamine (DA) as playing a role in the learned helplessness (LH) effect (1,5). Treatment with the tyrosine hydroxylase inhibitor α-methylparatyrosine (AMPT), which depletes DA, or with the dopamine antagonists haloperidol or pimozide caused naive mice to exhibit performance deficits comparable to those following inescapable stress (2). Administration of the DA precursor *l*-DOPA prevented escape deficits caused by AMPT (5) or by inescapable stress (4). The dopamine receptor agonist apomorphine also protected mice from the behavioral effects of inescapable stress whether administered prior to stress or after stress but prior to testing. Further experiments by the same group demonstrated that the behavior resulting after haloperidol administration could not be distinguished from that resulting from inescapable stress on the basis of response to noradrenergic agents (3), altered shock presentation, or novel cue presentation during testing (6). Although some of the pharmacological agents used in this early work were not specific for dopamine, the overall results suggested that dopamine blockade or depletion leads to subsequent development of a behavioral depression with many similarities to the learned helplessness produced by inescapable stress. Note that all the above experiments were performed with mice.

Dopamine involvement in other animal models of depression is also documented. In the behavioral despair or forced swim test, dopamine receptor blockade with haloperidol or sulpiride antagonized the behavioral effects of antidepressants (9), and the dopamine D_2 receptor agonist LY171555 had antidepressant-like effects (8) in this model.

In the recently developed chronic mild stress paradigm, dopamine receptor antagonists selectively reversed the improvement in performance caused by chronic antidepressant administration (20,28). Also, intermittent administration of the dopamine receptor agonists quinpirole and bromocriptine had antidepressant-like effects in this model that were reversed by raclopride, a D_2 antagonist. These results were interpreted as suggesting that the reversal of the behavior modeling depression was mediated by increased transmission at dopaminergic synapses (19).

We have previously postulated that the serotonin system in the medial prefrontal cortex (MPC) plays a central role in the

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development of learned helplessness (22,30). In the present work, we investigated whether haloperidol causes behavioral depression similar to stress-induced learned helplessness in the rat and whether this is anxiogenic. We also used in vivo microdialysis perfusion to study 5-HT release in MPC after intraperitoneal (IP) and intracortical (IC) administration of haloperidol, and after IC dopamine and apomorphine.

METHOD

Animals

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 200–250 g were used in all experiments. Rats were acclimated to the animal facility for at least a week, during which time they were housed in groups of six with ad lib access to chow and water. The protocol was approved by the Animal Study Committee of the Dallas VAMC and complies with the guidelines of the NIH.

Behavioral Experiments

Groups were randomly assigned to four experimental groups that received either vehicle (n = 17, 4 ml/kg) or haloperidol (5 mg/ml) at doses of 5, 10, or 20 mg/kg IP (n = 8, n = 13, n = 12, respectively). Twenty-four hours later each rat was tested for 5 min in the elevated plus maze using the procedure of Pellow et al. (21). Five minutes later, each rat was tested for learned helplessness with a shuttlebox using the procedure of Jackson et al. (14), later modified by Drugan et al. (13). Briefly, each trial began with an 80-dB tone followed in 5 s by a 1.0-mA grid shock. For the first five trials, shock could be terminated by one shuttle (FR1) and for the last 25 trials by two shuttles (FR2). If the rat failed to respond, shock terminated in 30 s. Trials were separated by a 60-s average variable interval. Thus, using the LH/NH criteria of Drugan et al. (13), rats with a mean latency of $\geq$20 s (of shock) for the 25 FR2 trials were considered LH.

Microdialysis Experiments

Microdialysis probes were constructed using the U-shaped design of Ungerstedt and Hallstrom (33) and were implanted under pentobarbital anesthesia into MPC using stereotaxic guidance at 4.2 A, 0.7 L, and 4.8 V relative to bregma. A day after probe implantation, perfusion was initiated using Ringer's solution, maintained at 1 ml/min. Microdialysis samples were collected at 20- or 30-min intervals and injected immediately onto a high performance liquid chromatography system equipped with a coulometric detector. Serotonin was quantitated with a sensitivity of 1 pg using an external standard (22). After obtaining a stable baseline, pharmacological treatments were administered as follows.

Experiment 1. Rats were injected IP with either lactate vehicle (4 ml/kg) or with haloperidol at 5, 10, or 20 mg/kg (n = 6 per group).

Experiment 2. After obtaining a stable baseline, perfusion was switched to Ringer's solution with 1 or 10 mM DA added (n = 3 per group).

Experiment 3. After obtaining a stable baseline, perfusion was switched to Ringer's solution with 1 mM apomorphine or 1 mM haloperidol in 3% propylene glycol (3% PG) added (n = 6 per group).

TABLE 1
ELEVATED PLUS MAZE

	Crossings Into Arms (mean)	Time Spent Closed Arms (s)
Controls (Vehicle)	8.7	112
Haloperidol (5 mg/kg)	8.8	116
Haloperidol (10 mg/kg)	5.6	151*
Haloperidol (20 mg/kg)	2.8*	168*

One-way ANOVA with Dunnett's post hoc test, *$p < 0.05$.

RESULTS

Behavioral Experiments

In the elevated plus maze (Table 1), rats receiving 10 and 20 mg/kg of haloperidol 24 h earlier spent significantly more time in the closed arms than rats receiving vehicle or haloperidol (5 mg/kg). Rats receiving the highest (20 mg/kg) dose of haloperidol had significantly fewer crossings into arms ($p <$ 0.05, ANOVA with Dunnett's post hoc test).

In the shuttlebox test for learned helplessness (Fig. 1), none of the haloperidol-treated groups had mean escape latencies for the first five FR1 trials significantly different from vehicle control. For the subsequent 25 FR2 trials, there was a group

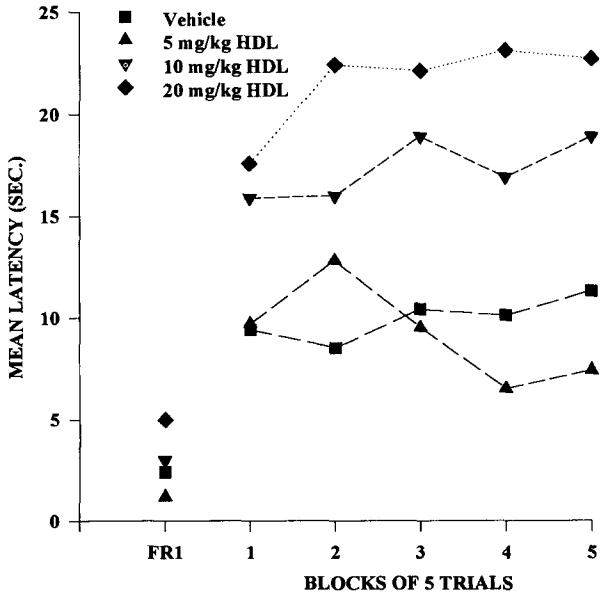

FIG. 1. Shuttlebox latency scores for haloperidol- or vehicle (control)-pretreated animals. Data presented are the mean ± SEM of the number of animals described in the text. Blocks 1–5 are FR2. Mean latencies for FR2 are significantly different by ANOVA, $F(3, 46) = 4.76, p = 0.006$.

TABLE 2
SHUTTLEBOX LATENCY

	Learned Helplessness	
	<20 s	≥20 s
Control (Vehicle)	13	4
Haloperidol (5 mg/kg)	8	0
Haloperidol (10 mg/kg)	7	6
Haloperidol (20 mg/kg)	4	8

$\chi^2 = 11.12, p < 0.01$.

difference by dose [ANOVA, $F(3, 46) = 4.76$, $p = 0.006$]. Dunnett's post hoc test at <0.05 found the 20-mg/kg dose to differ from control.

When rats were scored according to whether or not their escape latencies were in the normal (20 s) or learned helpless (≥20 s) range (Table 2), a dose-related increase in the proportion "helpless" was noted with increasing doses of haloperidol ($\chi^2 = 11.12, p < 0.01$).

Microdialysis Experiments

Experiment 1. Haloperidol (IP) at the 10-mg/kg dose increased 5-HT levels in perfusate of MPC by about 400% over baseline at 20 min after injection. This increase gradually dropped back to baseline during the next 2 h of perfusion. Increased 5-HT release was also observed after the 20-mg/kg dose of haloperidol to about 160% over baseline, but the 5-mg/kg dose was not significantly elevated over the saline control (Fig. 2). Statistical significance was determined with Mann-Whitney *U*-test ($p < 0.05$).

Experiment 2. Administration of DA directly into MPC via the microdialysis probe (reverse dialysis) increased 5-HT in perfusate from MPC at 10 μM but not at 1 μM concentration (Fig. 3) ($p < 0.05$, Mann-Whitney *U*-test).

Experiment 3. Both haloperidol and apomorphine significantly increased levels of 5-HT in perfusate from MPC when administered through the microdialysis probe at 1 mM ($p < 0.05$ vs. the appropriate control, Mann-Whitney *U*-test). Haloperidol produced a higher (350% vs. 170% of basal) level of 5-HT than apomorphine (Fig. 4).

DISCUSSION

To our knowledge, this is the first report that the DA blocking agent haloperidol causes behavior similar to stress-induced learned helplessness in the rat. This finding supports and extends similar work in mice (1). Results obtained were likely not due to sedative or motor effects of haloperidol because the drug was administered 24 h prior to behavioral testing. During testing, rats receiving haloperidol the day before appeared normal to inspection and handling. However, the best evidence that haloperidol's sedative or motor effects had dissipated prior to testing came from the fact that haloperidol-treated rats performed the FR1 shuttlebox trials similar to rats receiving saline. The learned helplessness inducing effects of haloperidol were clearly dose related, with 5 mg/kg having minimal effect. The highest dose of haloperidol (20 mg/kg) produced shuttlebox behavior comparable to that seen after inescapable shock using established procedures (22).

On the elevated plus maze there were fewer crossings into arms with the highest dose of haloperidol (20 mg/kg) and increased time spent in the closed arms at the 10- and 20-mg/kg doses of haloperidol. This effect is characteristic of anxiogenic drugs (21). An anxiogenic effect that correlates with

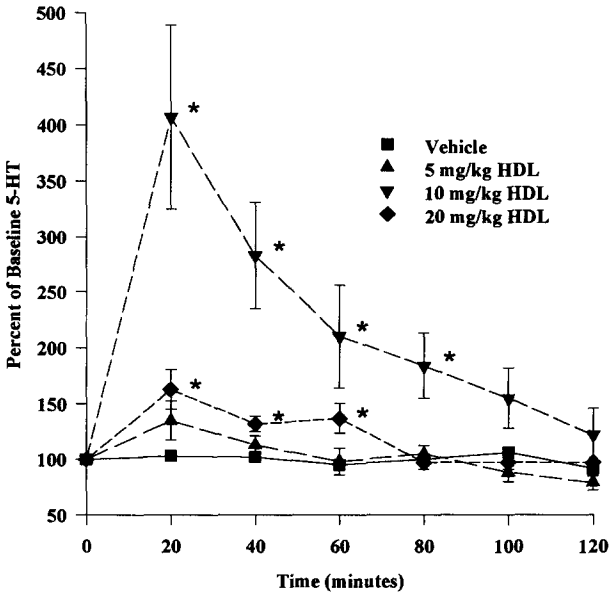

FIG. 2. The effect of haloperidol at doses of 5, 10, and 20 mg/kg on cortical 5-HT levels in microdialysis perfusate. The drug or vehicle was injected at $t = 0$. The mean ± SEM for five to six animals at each dose is presented. *$p < 0.05$ vs. control vehicle, Mann-Whitney *U*-test.

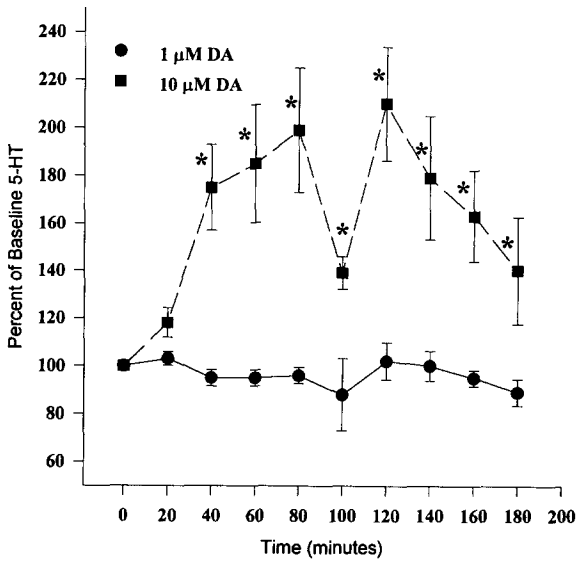

FIG. 3. The effect of dopamine infusion through the microdialysis probe on cortical 5-HT levels in microdialysis perfusate. Dopamine was introduced at $t = 0$. Mean 5-HT levels ± SEM for four animals in each group is presented. *$p < 0.05$, Mann-Whitney *U*-test.

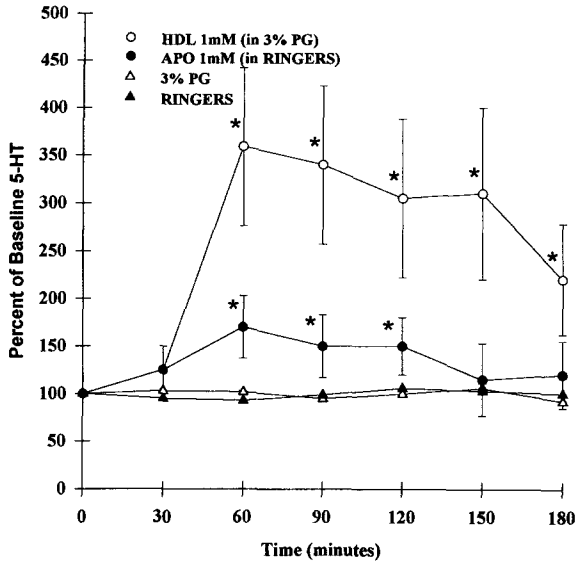

FIG. 4. The effect of perfused haloperidol (HDL), apomorphine (APO), or corresponding vehicle through the microdialysis probe on cortical 5-HT levels in microdialysis perfusate. The drug/vehicle was introduced at $t = 0$. Data represent the mean ± SEM for six animals in each group. $*p < 0.05$ vs. appropriate vehicle control, Mann-Whitney U-test.

learned helplessness is compatible with the previously reported finding that anxiogenic drugs produce learned helplessness (11). Because anxiolytics prevent learned helplessness (12,31), it is tempting to speculate that in the learned helplessness model an element of anxiety is necessary for the development of depression. There is a clinical correlation for this idea, because in prospective studies most cases of severe anxiety disorders develop major depression during follow up, and a clinical comorbidity between anxiety and depression is well documented (16,32).

Even at the highest dose tested, haloperidol administered 24 h prior to testing caused learned helplessness behavior in only 8/12 (67%) of rats (Table 2). This proportion is comparable to that seen when rats become helpless after inescapable stress. Since the first report that learned helplessness occurred after inescapable foot shock in 75% of dogs (29), a consistent finding has been that not all animals develop behavioral depression after inescapable stress. The present work makes it tempting to speculate that regardless of how helplessness is caused, only a certain proportion of subjects respond by becoming helpless or depressed. Future research with animal models should focus on identifying factors predisposing to this vulnerability.

Haloperidol increased in vivo 5-HT release from MPC. Our previous work has implicated the 5-HT system in MPC as central to the mechanism of antidepressant action in the learned helplessness model (30). Early in vivo experiments using push-pull perfusion suggested 5-HT depletion in FNC to correlate with subsequent development of learned helplessness (24). Recently, using in vivo microdialysis, we measured basal release of 5-HT before and after inescapable tail shock stress. Rats that became helpless after stress had increased basal release due to the stress compared to rats not helpless after stress or to nonshocked controls (23). In further studies we measured 5-HT levels in FNC perfusate from rats in which stress-induced helplessness had been prevented by either acute diazepam, subchronic imipramine, or behavioral training (22). In these experiments, we used a high K^+ perfusing medium to release intracellular 5-HT and found that depletion of K^+-released 5-HT correlated with development of helplessness. All three treatments preventing the behavioral depression also prevented the depletion of 5-HT. Therefore, it is plausible to speculate that a neurochemical mechanism involved in the development of learned helplessness, whether by stress or haloperidol, may be release of 5-HT from intracellular stores in FNC as measured by microdialysis of extracellular space.

However, there are two problems with this interpretation of the present work. First, the haloperidol-induced learned helplessness was dose related, with the greatest effect seen at 20 mg/kg, whereas the haloperidol induced 5-HT release was increased more at 10 mg/kg than at 20 mg/kg. There are several possible explanations for this, but additional research is needed to clarify this discrepancy. Second, the present research design is correlational; that is, different rats participated in the behavioral and in the neurochemical experiments. Future experiments should include a within-subject design in which haloperidol-induced 5-HT release can be directly correlated with helpless behavior in individual subjects.

The primary neurochemical effects of haloperidol are usually considered to be mediated via DA receptor blockade. Interactions between DA and 5-HT are well documented, particularly at subcortical levels (34). Our results suggest a possible cortical interaction as well, because perfusing haloperidol directly into FNC caused increased 5-HT release. Systemic administration of haloperidol increases in vivo DA release in MPC (18). Also, increased DA activity in MPC appears to be a ubiquitous consequence of stress (7). Stress, of course, is a prerequisite for behavioral helplessness. Therefore, it is of some interest that we now report that perfusing DA itself into MPC caused increased 5-HT release. Similarly, the DA

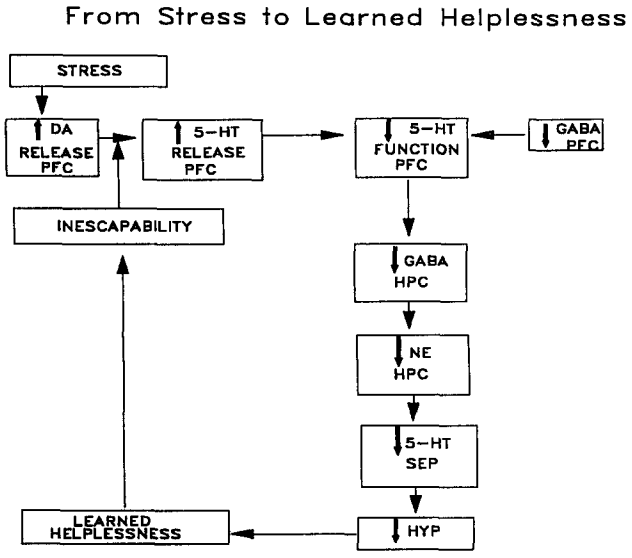

FIG. 5. Hypothetical map of biochemical and neuroanatomical effects of inescapable stress. Abbreviations: MPC = medial prefrontal cortex, HPC = hippocampus, SEP = septum, HYP = hypothalamus, DA = dopamine, 5-HT = serotonin, NE = norepinephrine.

agonist apomorphine also increased 5-HT release when infused into MPC. Thus, one explanation for the data is that haloperidol, because of its postsynaptic D_2 receptor blockade, causes a compensatory increased presynaptic DA release in MPC, and that DA itself, perhaps acting on a non-D_2 receptor, induces increased 5-HT release in MPC that in turn leads to learned helplessness. Another explanation is that haloperidol interacts directly with a 5-HT receptor regulating 5-HT release, and indeed haloperidol binds to the 5-HT_2 receptor with high affinity (17).

We may use the present data and previous information to expand the hypothetical neurochemical map (30) we have proposed for learned helplessness (Fig. 5). The new version incorporates a role for DA in medial prefrontal cortex as a common feature of stress. Probably cortical DA release is one way to explain how stress leads to helplessness in some subjects. In this context it is interesting that DA depletion in MPC did not prevent rats from becoming helpless after foot shock (26), suggesting other neurochemical pathways to be involved. Also, it is possible that the physiological effects of helplessness or stress-induced behavioral depression are themselves sufficiently stressful to perpetuate a maladaptive behavior after the initial stress has ceased. In this model, dopamine contributes to the neurochemistry of stress-induced behavioral depression as part of a complex regional neuronal interaction involving several neurotransmitters (Fig. 5), specifically a serotonin locus in medial prefrontal cortex and septum, and a GABA/norepinephrine interaction in hippocampus. A relationship between dopamine and learned helplessness, and between dopamine and human depression (10, 15,25,27), has been postulated and the present work supports this connection.

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