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# Inhibition of amine uptake by 4-phenyl-bicyclo(2,2,2)octan-1-amine hydrochloride monohydrate (EXP 561) in rats

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4-Phenyl-bicyclo(2,2,2)octan-1-amine hydrochloride monohydrate (EXP 561) is an experimental drug that inhibits amine uptake [1, 2] and has various pharmacologic effects expected of an amine uptake inhibitor and/or an antidepressant drug in animals [3, 4]. In studying the effects of various compounds on amine uptake *in vivo*, we have observed that EXP 561 is a remarkably potent inhibitor of uptake into serotonin neurons and norepinephrine neurons in rats. To evaluate uptake inhibition *in vivo*, we measured antagonism of monoamine depletion by depleting drugs that require active uptake into the neuron. *p*-Chloroamphetamine, a depletor of serotonin in brain, requires active uptake as indicated by the ability of uptake inhibitors to block its depleting effect on serotonin [5, 6]. 6-Hydroxydopamine is actively taken up into norepinephrine neurons and there depletes norepinephrine; its effects are also blocked by appropriate amine uptake inhibitors [6, 7].

In our experiments, male Wistar rats weighing about 150 g were used. *p*-Chloroamphetamine hydrochloride (Regis) was injected i.p. at a dose of 10 mg/kg 4 hr before the rats were killed, and 6-hydroxydopamine hydrobromide (Regis) was injected i.p. at a dose of 100 mg/kg 16 hr before the rats were killed. EXP 561 (Dupont) was administered to some rats by i.p. injection 1 hr before the depleting drugs. In some experiments, iprindole (Wyeth) was injected i.p. at 10 mg/kg 1 hr before EXP 561. Rats were killed by decapitation, and whole brains were rapidly removed and frozen on dry ice, then stored frozen prior to analysis. Serotonin and norepinephrine levels were assayed spectrofluorometrically [8]. Levels of EXP 561 were measured spectrophotometrically by reaction with methyl orange [9]. This colorimetric method was sensitive to approximately 0.2 $\mu$g EXP 561. Since recovery of EXP 561 was only 40 per cent, internal standard curves with known amounts of drug added to tissue homogenates were used to calculate EXP 561 concentrations in tissue. There were five rats per experimental group, and all data are expressed as mean values $\pm$ standard errors. Comparisons between groups were made by the Student *t*-test.

Figure 1 shows the ability of EXP 561 to block the depletion of brain serotonin by *p*-chloroamphetamine and of heart norepinephrine by 6-hydroxydopamine in rats. A dose of 1 mg/kg of EXP 561 almost completely blocked the depletion of brain serotonin, whereas the 0.1 mg/kg dose was without effect. The observation that EXP 561 blocks uptake into serotonin-containing neurons in rat brain *in vivo* extends the work of Campbell and Todrick [2] who showed that it inhibited serotonin uptake into platelets *in vitro*. The dose–response curve for 6-hydroxydopamine antagonism by EXP 561 was less steep; the 0.1 mg/kg dose caused significant antagonism but even the 10 mg/kg dose did not completely prevent the effect of 6-hydroxydopamine. Nonetheless the 1 mg/kg dose produced almost 50 per cent antagonism, which means that EXP 561 is a potent inhibitor of uptake into norepinephrine-containing neurons as well as into serotonin-containing neurons. This finding complements the data of Sarges *et al.* [1], who showed that EXP 561 antagonized uptake of [$^3$H]norepinephrine into rat heart *in vivo*.

EXP 561 has some structural resemblance to amphetamine in having a phenyl group and a primary amine group separated about the same distance by aliphatic carbons. Rats are known to metabolize amphetamine primarily by hydroxylation in the para position of the phenyl ring [10]. This metabolic route is blocked by iprindole [11] and various other drugs [12]; as a consequence, tissue levels of amphetamine are increased, its half-life is prolonged, and various pharmacologic effects of amphetamine are potentiated. We therefore determined if iprindole would

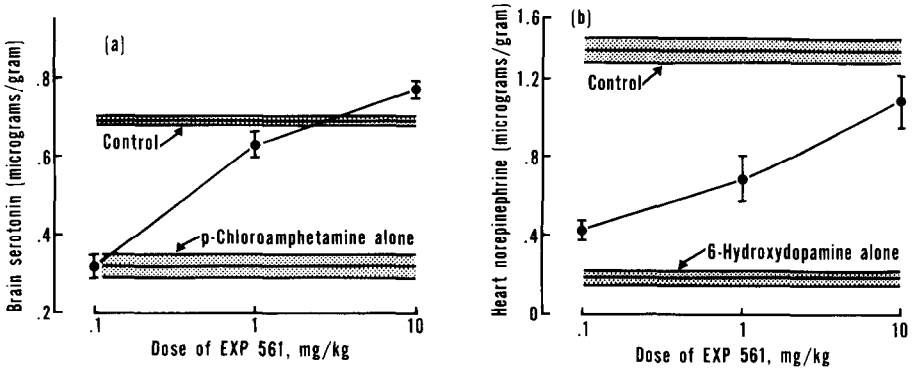


Fig. 1. Antagonism by EXP 561 of (a) brain serotonin depletion by *p*-chloroamphetamine and (b) heart norepinephrine depletion by 6-hydroxydopamine as indices of uptake inhibition into serotonin and norepinephrine neurons in rats. Horizontal lines and shaded areas represent mean and standard error range for control rats or rats treated with depleting agents alone. Dots represent mean values for rats treated with EXP 561 prior to depleting agent.

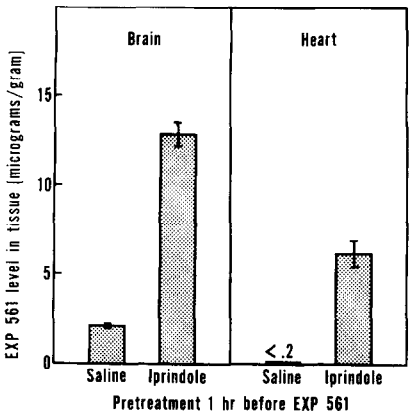


Fig. 2. Concentration of EXP 561 in brain and heart as influenced by iprindole pretreatment in rats. EXP 561 (10 mg/kg) was injected 1 hr before rats were killed and 1 hr after saline or iprindole (10 mg/kg).

affect tissue levels of EXP 561 and degree of uptake inhibition by EXP 561. Figure 2 shows that the levels of EXP 561 in heart and in brain were markedly increased by iprindole pretreatment. As a consequence, the ability of EXP 561 to antagonize *p*-chloroamphetamine and 6-hydroxydopamine was increased. In iprindole-pretreated rats, antagonism of *p*-chloroamphetamine-induced depletion of brain serotonin was 4, 30, 48 and 93 per cent, respectively, after doses of EXP 561 of 0.01, 0.025, 0.05 and 0.1 mg/kg. Thus, a dose of 0.05 mg/kg produced about 50 per cent antagonism of serotonin depletion, compared to 0.5 mg/kg estimated by interpolation with the data in Fig. 1 for rats not pretreated with iprindole. Antagonism of 6-hydroxydopamine-induced depletion of heart norepinephrine in iprindole-pretreated rats was 12, 43, 44, 68 and 69 per cent, respectively, after doses of 0.05, 0.1, 0.25, 0.5 and 1 mg/kg of EXP 561. Interpolation gave extimated $ED_{50}$ values of 0.3 mg/kg and 2 mg/kg from these data and those in Fig. 1 for EXP 561 with and without iprindole pretreatment respectively. Iprindole itself did not antagonize *p*-chloramphetamine or 6-hydroxydopamine, as expected from the lack of activity of iprindole as an uptake inhibitor [13].

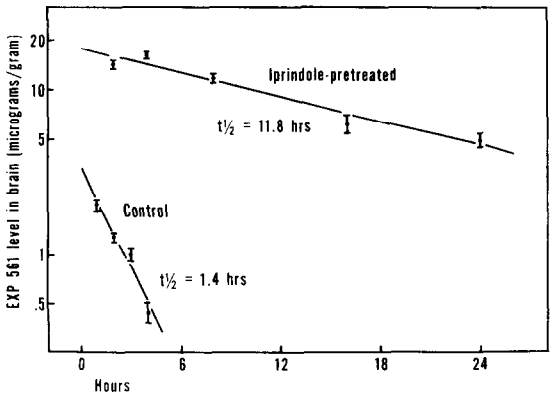


Fig. 3. Disappearance of EXP 561 from brain of control or iprindole-pretreated rats. EXP 561 (10 mg/kg) was injected at zero time into control rats or rats pretreated 1 hr earlier with iprindole (10 mg/kg). Half lives were calculated from least squares calculation of lines best fitting the data points.

To determine quantitatively the extent of iprindole's influence on EXP 561 removal from tissues, we measured brain half-lives of EXP 561 with and without iprindole pretreatment (Fig. 3). In control rats, EXP 561 disappeared from whole brain with a half-life of 1.4 hr, similar to the half-life for amphetamine [14]. Iprindole pretreatment markedly increased EXP 561 levels in brain and prolonged the half-life of EXP 561 to 11.8 hr. These results suggest that iprindole pretreatment interferes with the metabolism of EXP 561. Although we have no direct proof that the metabolic step being inhibited is ring hydroxylation, that possibility seems likely in view of the known ability of iprindole to inhibit the ring hydroxylation of amphetamine [11].

In summary, we have obtained data showing that EXP 561 is a potent inhibitor of uptake both into serotonin-containing and norepinephrine-containing neurons in rats. The potency of EXP 561 is increased still further by iprindole pretreatment. Iprindole pretreatment increased EXP 561 levels in brain and heart and prolonged its half-life, giving suggestive indirect evidence that EXP 561 may be metabolized in rats by ring hydroxylation in a manner similar to the metabolism of amphetamine.

*The Lilly Research Laboratories,* RAY W. FULLER
*Eli Lilly & Co.,* HAROLD D. SNODDY
*Indianapolis, Ind.* 46206, *U.S.A.* KENNETH W. PERRY

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