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EFFECT OF A BUTYROPHENONE DERIVATIVE, U-32,802A, ON BRAIN BIOGENIC AMINES

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Abstract—U-32,802A, a new butyrophenone derivative, was found to block the uptake of and to cause a release of ^{3}H-norepinephrine from the mouse heart. The releasing effect of U-32,802A was not blocked by pretreatment with protriptyline at 10 mg/kg. U-32,802A caused a severe and long-lasting depletion of mouse brain norepinephrine and dopamine with little effect on serotonin at a dose of 5 mg/kg i.p. U-32,802A also caused an increase in homovanillic acid in mouse striatum, a property common to a variety of antipsychotic agents. The spectrum of activity of U-32,802A is different from that of either haloperidol or reserpine and may represent a new class of amine-depleting agents.

U-32,802A, 4′-FLUORO-4-[[4-(*p*-fluorophenyl)-3-cyclohexen-1-yl]-amino]-butyrophenone, hydrochloride is a new butyrophenone derivative of which haloperidol may be considered the prototype. This report describes some of the biochemical–pharmacological properties of this compound. Previous papers have described the chemistry and basic pharmacology of this new class of butyrophenones.[1,2]

EXPERIMENTAL

Male Carworth Farm mice, 18–20 g, were used as well as Upjohn Sprague–Dawley rats weighing 125–150 g. Studies on the uptake and release of (DL)-^{3}H-norepinephrine (New England Nuclear; sp. act., 6·5 Ci/m-mole) from the mouse heart were carried out as previously described,[3] as were the studies on the uptake of ^{14}C-serotonin (New England Nuclear; sp. act., 26·7 mCi/m-mole) by the mouse spleen;[4] each mouse was given 1·5 μCi(DL)-^{3}H-norepinephrine and 0·125 μCi ^{14}C-serotonin.

Endogenous norepinephrine, dopamine and serotonin were extracted and measured by previously described methodology,[5] as was homovanillic acid.[6]

RESULTS

Initial studies showed that U-32,802A at a screening dose of 10 mg/kg i.p. was very effective in blocking the uptake of ^{3}H-norepinephrine (^{3}H-NE) into the mouse heart (8 per cent of control) and also in blocking the uptake of ^{14}C-serotonin (^{14}C-5-HT) into the mouse spleen (49 per cent of control) (Table 1). Further studies demonstrated that U-32,802A could also cause a release of ^{3}H-NE from the mouse heart at 1 mg/kg (43 per cent of control) and that this releasing effect was not antagonized by protriptyline at 10 mg/kg (Table 2).

The uptake and release studies were followed up by an examination of the effect of U-32,802A on whole mouse brain amines. The data, which are presented in Table 3, show that U-32,802A exerted a pronounced effect on mouse brain NE and DA

TABLE 1. EFFECT OF U-32,802A ON THE UPTAKE OF ^{3}H-NE AND ^{14}C-5-HT*

Dose (mg/kg)	Mouse heart ^{3}H-NE (cpm/1·5 ml)	(% Control)	Mouse spleen ^{14}C-5-HT (cpm/1·5 ml)	(% Control)
	8130 ± 845		4064 ± 260	
10 (i.p.)	655 ± 156	8	1991 ± 156	49

* ^{3}H-NE (1·5 μCi) and ^{14}C-5-HT (0·125 μCi) were administered i.v. 1 hr after drug or vehicle and all mice were sacrificed 3 hr after ^{3}H-NE and ^{14}C-5-HT. Three determinations were obtained for each drug and dose, and two mice were used per determination.

TABLE 2. EFFECT OF U-32,802A ON THE RELEASE OF ^{3}H-NE FROM THE MOUSE HEART*

Drug	Dose (mg/kg)	Mouse heart ^{3}H-NE (cpm/1·5 ml)	(% Control)	P vs control
Vehicle		9579 ± 186	100	
Protriptyline	10	10,375 ± 503	108	NS (at P = 0·01)
U-32,802A	1	4145 ± 548	43	P < 0·01
Protriptyline + U-32,802A	10 + 1	4924 ± 215	51	P < 0·01

* Protriptyline was given 1 hr after ^{3}H-NE (1·5 μCi); U-32,802A was administered 1·5 hr after ^{3}H-NE and all mice were sacrificed 3 hr after ^{3}H-NE. Two mice were used per determination and three determinations were made per schedule.

TABLE 3. EFFECTS OF U-32,802A ON MOUSE BRAIN AMINES

Dose (mg/kg)	NE (μg/g ± S.E.M.)	DA (μg/g ± S.E.M.)	5-HT (μg/g ± S.E.M.)
Vehicle	0·41 ± 0·02	0·89 ± 0·05	0·51 ± 0·02
1·0	0·23 ± 0·01*	0·45 ± 0·09*	0·62 ± 0·03
3·0	0·08 ± 0·01*	0·15 ± 0·02*	0·45 ± 0·03
10·0	0·06 ± 0·01*	0·09 ± 0·01*	0·45 ± 0·03

* Significant at P = 0·01 (Student's *t*-test). Mice were sacrificed 3 hr after i.p. drug administration. Two brains were used per determination and five determinations were made per treatment.

TABLE 4. TIME STUDY OF EFFECT OF U-32,802A ON MOUSE BRAIN CATECHOLAMINES*

Sacrifice time (hr)	NE (μg/g ± S.D.)	DA (μg/g ± S.D.)
	0·48 ± 0·13	1·20 ± 0·15
8	0·02 ± 0·02	0·08 ± 0·04
24	0·04 ± 0·03	0·11 ± 0·04
48	0·06 ± 0·04	0·08 ± 0·04
72	0·25 ± 0·06	0·58 ± 0·15

* Mice were administered 5 mg/kg of U-32,802A (i.p.) and sacrificed at the designated times. Two brains were used per determination and three determinations were made per period.

(dopamine) and very little effect on 5-HT (serotonin) at doses of 1, 3 and 10 mg/kg i.p.

A time study (Table 4) further showed that U-32,802A at 5 mg/kg not only produces a large decrease in the amine levels but also exerts a long-lasting effect. Near total depletion of NE and DA was observed at 8 hr and recovery toward normal did not occur until 72 hr.

TABLE 5. EFFECTS OF U-32,802A, HALOPERIDOL AND RESERPINE ON RAT BRAIN AND HEART BIOGENIC AMINES*

		Brain			Heart
Drug	Dose (mg/kg)	NE (μg/g ± S.E.M.)	DA (μg/g ± S.E.M.)	5-HT (μg/g ± S.E.M.)	NE (μg/g ± S.E.M.)
Vehicle		0·44 ± 0·02	0·97 ± 0·03	0·46 ± 0·02	0·55 ± 0·13
Reserpine	3	0·04 ± 0·01	0·06 ± 0·02	0·12 ± 0·03	0·02 ± 0·01
Haloperidol	30	0·31 ± 0·02	0·69 ± 0·07	0·40 ± 0·03	0·70 ± 0·09
U-32,802A	3	0·16 ± 0·01	0·36 ± 0·02	0·30 ± 0·02	0·38 ± 0·05

* Rats were sacrificed 4 hr after the i.p. drug administration. One brain or heart was used per determination and four determinations were made per drug.

The effect of U-32,802A on rat brain and heart amines at a dose of 3 mg/kg was studied and compared to the effects of reserpine at 3 mg/kg and haloperidol at 30 mg/kg. In this study (Table 5) U-32,802A was found to be less active than reserpine in the degree of amine depletion and especially so with respect to its effect on brain serotonin and heart norepinephrine. Haloperidol, at this extremely high dose, caused a 30 per cent reduction in brain norepinephrine and dopamine, and had essentially no effect on serotonin.

TABLE 6. EFFECT OF U-32,802A AND HALOPERIDOL ON HOMOVANILLIC ACID IN THE MOUSE STRIATUM*

Drug	Dose (mg/g)	Homovanillic acid (μg/g ± S.E.M.)	P vs control
Vehicle		0·15 ± 0·05	
U-32,802A	2·0	0·26 ± 0·03	$P < 0.05$
U-32,802A	4·0	0·41 ± 0·08	$P < 0.05$
U-32,802A	8·0	0·47 ± 0·08	$P < 0.05$
Haloperidol	0·25	0·45 ± 0·01	$P < 0.05$

* Striatal tissue from four mice was used per determination and three determinations were made per schedule. Drugs were administered by the i.p. route and all mice were sacrificed 2 hr later.

A property characteristic of haloperidol and the phenothiazine antipsychotic agents is their ability to cause an elevation in striatal HVA.[6] The data presented in Table 6 demonstrate that U-32,802A is capable of causing an elevation in striatal HVA at doses of 2, 4 and 8 mg/kg. Haloperidol was found to be considerably more active than U-32,802A in producing an elevation in HVA.

DISCUSSION

The results of this series of studies have defined the biochemical activities of a new butyrophenone derivative, U-32,802A. This compound was found to be active in blocking the uptake of ^{3}H-NE into the mouse heart and it also proved to be effective in releasing ^{3}H-NE from the mouse heart. Protriptyline has been shown to block the norepinephrine-depleting capabilities of guanethidine, 6-hydroxydopamine and metaraminol in the mouse heart, whereas it did not block the effect of reserpine in this system.[7] In this test it was found that pretreatment with protryptyline at 10 mg/kg did not prevent the releasing action of U-32,802A on heart ^{3}H-NE and such data would place U-32,802A in a category of amine depletors similar to that of reserpine.

The amine-depleting activity of U-32,802A was further exemplified by its effects on whole mouse brain amines (Table 3). A 50 per cent decrease in NE and DA was seen with 1 mg/kg and almost total depletion occurred at 3 mg/kg within 3 hr. Serotonin was depleted to only a minor extent at the 10 mg/kg dose of U-32,802A. This minimal effect of U-32,802A on brain serotonin points out a difference between it and reserpine. U-32,802A, at 5 mg/kg, produced a severe and long-lasting (48 hr) depletion of NE and DA, and a return toward normal was observed at 72 hr after drug administration.

The studies in the rat supported, to a great extent, the data obtained in mice. U-32,802A at 3 mg/kg caused a 65 per cent decrease in brain NE and DA and a 35 per cent decrease in 5-HT. Heart NE was not depleted to nearly the same extent as was brain NE, 65 vs 30 per cent depletion. Reserpine, in contrast to U-32,802A, produced a near maximal depletion of all three brain amines and heart NE. Haloperidol, at the extremely high dose of 30 mg/kg, produced only negligible effects on the brain amines.

Consistent with the effects of haloperidol and the phenothiazine antipsychotics, U-32,802A caused a significant increase in mouse striatal HVA at 2 mg/kg (Table 6). The elevation in HVA has been associated with both the antipsychotic activity of these compounds and their ability to produce extrapyramidal side effects in man.[8]

The net result of these studies indicates that U-32,802A has a potent and long-lasting depleting effect on brain catecholamines. The inability of protryptyline pretreatment to block its ^{3}H-NE releasing property places U-32,802A in a category similar to reserpine; however, its weak depleting activity on brain serotonin separates it from reserpine. Haloperidol exerts little effect on brain biogenic amine levels and is a potent elevator of striatal HVA. In contrast, U-32,802A is a catecholamine depletor and it also elevates striatal HVA. Therefore it appears that U-32,802A has a spectrum of activity different from that of either haloperidol or reserpine and may represent a new class of catecholamine-depleting agents and potential antipsychotic drugs.

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