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ACTION OF THE ANTIDEPRESSANT PRIDEFINE (AHR-1118) ON BIOGENIC AMINES IN THE RAT BRAIN

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Abstract—The antidepressant efficacy of pridefine has been established in previous clinical trials. The present study was undertaken to determine its mode of action. Regional levels of norepinephrine, dopamine and 5-hydroxytryptamine were measured following pridefine administration in rats pretreated with saline or reserpine. Only hypothalamic levels of norepinephrine were increased significantly in both control and reserpinized rats. *In vitro* effects of pridefine on transmitter uptake and release were examined. Uptake blockade predominated but some releasing activity was present, leading to the conclusion that the drug is a reuptake blocker. *Ex vivo* uptake experiments confirmed the results of *in vitro* studies and indicated that the drug reaches active sites.

Although tricyclic antidepressants and monoamine oxidase (MAO) inhibitors are the established pharmacotherapeutic agents for the treatment of depression, pharmacologists and clinicians continue to seek new compounds with fewer contraindications and a more rapid onset of clinical action. Pridefine, (1-ethyl-3-diphenylmethylenepyrrolidine) hydrochloride (AHR-1118), may be one such compound. In an early comparative study with amitriptyline, pridefine caused less sedation, was more effective in alleviating psychomotor retardation, and appeared to have an earlier onset of antidepressant action [1]. A recent double-blind study with patients suffering from primary affective disorder and alcoholism [2] and an open clinical study of patients with major depressive disorder [3] have shown that the drug is clinically as efficacious as imipramine and less toxic than standard antidepressants. The pharmacological basis for the clinical efficacy of pridefine was not apparent, however, due to the lack of structural resemblance with commercially available psychotropic compounds (Fig. 1).

In this study the biochemical pharmacological properties of pridefine were evaluated in order to characterize its mode of action. Because the drug does not inhibit MAO†, variables related to the mode of action of tricyclic antidepressants were measured. Previous work in this laboratory had demonstrated time-dependent increases in brain levels of biogenic amines following administration of tricyclic antidepressants [4, 5]. An initial aim was to determine whether pridefine selectively alters regional levels of norepinephrine (NE), dopamine (DA) or 5-hydroxytryptamine (5-HT). Amine levels were measured at two intervals after drug was given to animals pretreated with saline or reserpine. Since changes in transmitter levels imply altered neuronal activity, the second part of the study focused on the mechanism by which pridefine may act at the neuronal level. Numerous studies have shown that tricyclic antidepressants act as reuptake blockers and, to a lesser extent, as releasing agents [6]. It was determined, therefore, whether pridefine blocks the *in vitro* uptake of NE, DA and 5-HT and whether selective inhibition occurs with a particular transmitter and/or brain region. To assess the physiological significance of *in vitro* uptake inhibition, *ex vivo* uptake was measured in synaptosomal preparations from rats pretreated with pridefine. Finally, a series of release experiments were conducted.

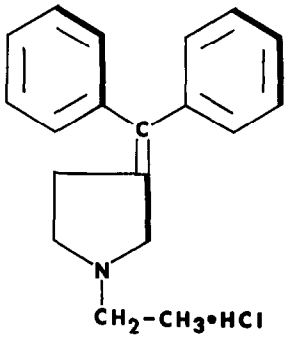

Fig. 1. Structural formula of pridefine (AHR-1118).

MATERIALS AND METHODS

Materials. Male Sprague–Dawley rats, 150–200 g in weight, were supplied by King Laboratories, Oregon, WI. Animals were maintained at constant temperature and relative humidity with a 12-hr light–dark cycle. Food and water were available *ad lib.* All animals were decapitated between 10:00 a.m. and 12:00 p.m. Where applicable, dissections of hypothalamus, striatum, midbrain, hindbrain, frontal cortex and nucleus accumbens were performed immediately after decapitation as described by Halaris *et al.* [7].

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Pridefine was provided by the A. H. Robins Co. (Richmond, VA). Reserpine (Serpasil) and reserpine placebo were supplied by the Ciba-Geigy Corp. (Summit, NJ). The following compounds were obtained from the New England Nuclear Corp. (Boston, MA): [^{3}H]dihydroxyphenylethylamine (sp. act. 6.45 Ci/mmole), [^{3}H]norepinephrine (sp. act. 8.72 Ci/mmole), [^{3}H]-5-hydroxytryptamine binoxalate (sp. act. 29.2 Ci/mmole), [^{3}H]-*S*-adenosyl-*l*-methionine (sp. act. 8–10 Ci/mmole), aquasol scintillation counting fluid, and omnifluor. Soluene 350 was purchased from the Packard Instrument Co. (Downers Grove, IL), and dopamine–HCl, *l*-norepinephrine–HCl, serotonin creatinine sulfate, 3-methoxytyramine, normetanephrine, tetraphenylboron, *d,l*-dithiothreitol and ouabain octahydrate were obtained from the Sigma Chemical Co. (St. Louis, MO). Dihydroxybenzylamine (DBHA) was supplied by the Aldrich Chemical Co. (Milwaukee, WI). All other reagent grade chemicals were obtained through local distributors.

Measurement of NE, DA and 5-HT levels. Rats were injected i.p. with reserpine (5 mg/kg), reserpine placebo or saline in equivalent volumes. To assess the degree of reserpinization, the weight loss criterion of Halaris and Freedman [8] was employed. Rats were weighted 24 hr following administration of reserpine; those that did not exhibit a 5 per cent or more loss of body weight were excluded from the study. Of the animals used, the mean per cent weight loss was *ca.* 15.2 per cent. Rats then received pridefine (25 mg/kg, i.p.) or saline in equivalent volume. Following administration of the drug, their behavior was observed until they were killed at 30 or 60 min.

Brain regions were homogenized in appropriate volumes of ice-cold 0.1 N perchloric acid with 0.1% EDTA (striatum, 5.0 ml; hypothalamus, 2.0 ml; frontal cortex, 1.0 ml; and nucleus accumbens, 1.5 ml). Homogenates were centrifuged at 40,000 *g* for 35 min. The protein-free supernatant fractions were stored at −80° for no more than 96 hr before assay.

The radioenzymatic method of Coyle and Henry [9] as modified by Daprada and Zurcher [10] was used to measure catecholamine levels. Levels of 5-HT were determined by high-pressure liquid chromatography with electrochemical detection (h.p.l.c.–e.c.). Chromatographic conditions were similar to those of Sasa and Blank [11] except that a 500 × 2 mm column was substituted for the longer column used in their study. In addition, a pneumatic pump [12] was substituted for a reciprocating pump, and the flow was lowered to 0.4 ml/min. The mobile phase consisted of 8.2 g sodium acetate, 4.25 g NaOH, 10.5 g citric acid, 2.1 ml glacial acetic acid, and 1 liter of distilled deionized water. Immediately prior to use, the mobile phase was filtered through a 0.45 μm millipore filter and degassed under vacuum for *ca.* 2 hr. Following degassing, the pH was carefully adjusted to pH 5.1 with glacial acetic acid and loaded into the pump reservoir under vacuum.

A 25-μl aliquot of DHBA was added to 0.5 ml of tissue homogenate. To prevent precipitation of perchlorate crystals onto the column, the pH of the samples was adjusted to the 5.1–6.0 range with aliquots of the mobile phase and 0.5 N KOH. Samples were placed on ice for at least 30 min to facilitate precipitation and then were filtered through CF50A amicon conical membrane filters (Amicon Corp., Lexington, MA) at 900 *g* for 45 min.

Twenty microliters of sample was applied with a sliding loop injector (Altex) to a glass column packed with Dupont SCX resin and run at room temperature. Samples were amperometrically detected at 0.6 V electrode potential using an LC-2A detector and glassy carbon working electrode (Bioanalytical Systems, Lafayette, IN). Peak identification, integration and quantification were performed by an SP4000 computing integrator (Spectra Physics, Santa Clara, CA). Areas of 5-HT peaks were quantified by the internal standard method using DHBA as the internal standard. Samples were compared to external calibration standards of authentic DHBA and 5-HT that were run with each analysis. Calibration standards were run at least six times, whereas experimental samples were run in triplicate.

Uptake of tritiated DA, NE and 5-HT. Synaptosomal uptake of tritiated DA, NE and 5-HT was measured by a modification of earlier methods used by Snyder and Coyle [13] and Halaris *et al.* [14]. Whole brain, frontal cortex, midbrain and hindbrain were homogenized in 10 vol. of ice-cold 0.32 M sucrose, whereas the hypothalamus and striatum were homogenized in 13.5 and 40 vol., respectively. Homogenates were centrifuged at 1000 *g* for 10 min, and the supernatant fractions were decanted for uptake and protein assays.

Krebs–Henseleit bicarbonate buffer (pH 7.4) was used in the incubation medium with DA and NE, whereas Krebs–Ringer phosphate buffer (pH 7.4) was substituted when 5-HT was measured. Except as noted, labeled substrates were added to the buffer to give a final concentration of 0.05 μM in 4 ml. Final drug (pridefine) concentrations varied between 3×10^{-7} and 1×10^{-5} M. Quadruplicate samples for controls and each drug concentration, consisting of 3.7 ml buffer, 0.1 ml drug or 0.1 ml water, were prepared for incubation. During the addition of 0.2-ml aliquots of synaptosomal suspension, the tubes were kept on ice to minimize uptake prior to incubation. The drug was added to the incubation mixture at the same time as the substrate and no preincubation was carried out. It had been established in this laboratory that results are similar with or without preincubation of the synaptosomal suspension with drug. Tubes were then transferred to a 37° water bath and agitated for 8 min. The incubation conditions were selected to reflect initial conditions, i.e. uptake was linear with respect to time. The incubation was terminated by transfer to an ice bath for 2 min, followed by centrifugation at 6000 *g* for 20 min. The pellet was rinsed with ice-cold saline and solubilized by incubation with 1.0 ml Soluene 350 at 50° for 30 min. Samples were transferred to glass counting vials containing 10 ml toluene–omnifluor and were measured in an Isocap 300 scintillation spectrometer. Except where noted, all samples were corrected for the effects of diffusion and substrate binding in order to determine active uptake.

Measurement of passive uptake. Total passive uptake (including binding and diffusion) was meas-

ured by inclusion of ouabain in the incubation mixture. This approach was chosen over the commonly used 'zero degree controls' for determination of passive uptake because ouabain is a potent and specific inhibitor of sodium–potassium-dependent ATPase, an enzyme involved in active uptake of monoamines [15]. A series of preliminary investigations were conducted to verify the usefulness and validity of ouabain in determining passive uptake. A detailed analysis of the effects of ouabain on substrate uptake and binding will be reported elsewhere.

Measurement of substrate binding. Substrate binding was measured in the same way as passive uptake except that tissue suspensions were ultrasonically disrupted upon termination of the uptake incubation and prior to pelleting. An ultrasonic cell disruptor model W-185 (Heat Systems Ultrasonics, Plainview, NY) was used, and tissue suspensions were sonicated with three consecutive 15-sec bursts at 40 W. Substrate binding was corrected for the effects of ouabain as will be reported in detail elsewhere.

Release of tritiated DA, NE and 5-HT. Release measurements were similar to those described for uptake except that the pellet collected from the first incubation was resuspended in the appropriate buffer and reincubated at 37° for 20 min. The second incubation was terminated by centrifigation at 6000 *g* for 20 min. The samples were then collected and counted as described for uptake. For both uptake and release experiments, proteins were measured by the method of Lowry *et al.* [16].

Calculations. A major aim of the present study was to examine the effects of pridefine on active synaptosomal uptake processes. Such processes are by definition saturable with respect to substrate concentration and, in fact, this condition is a prerequisite for justification of the application of data reduction techniques based on Michaelis–Menten kinetics. It was necessary, therefore, to correct the observed uptake velocities for the contribution of nonsaturable (passive) components, e.g. diffusion and binding. Active uptake was calculated by subtracting the values obtained for the passive component (procedure described above) from the observed uptake measurements.

The effectiveness of drug-induced uptake inhibition and the releasing potencies (IC_{50} and RC_{50}) were determined by log–logit analysis [17]. Calculations were restricted to those drug concentrations that gave between 20 and 80 per cent inhibition or release of the maximally observed effect.

Results were evaluated statistically by the *t*-test using an HP-33E programmable calculator (Hewlett Packard, Cupertino, CA) and programs recommended by the manufacturer.

RESULTS

Amine levels and reserpinization. Levels of NE, DA and 5-HT were measured in the hypothalamus, striatum, frontal cortex and nucleus accumbens. Pridefine significantly elevated levels of hypothalamic NE (Table 1). In unpretreated rats, NE levels had increased 22 per cent at 30 min after injection. The NE in reserpinized animals with 90 per cent amine depletion had increased 59 per cent at 30 min after pridefine. There were no significant changes in NE levels in either control or pretreated rats decapitated 60 min following drug. At 60 min, values were nearly identical to those obtained with saline, and indicate that, following a single dose of pridefine, NE levels increased around 30 min and rapidly declined to control levels. No statistically significant changes in NE levels occurred in the frontal cortex at either 30 or 60 min post-drug. Similarly, values obtained at both 30 and 60 min in reserpinized rats were not altered. For both regions, NE levels measured in control animals were comparable to those obtained by other investigators [18–20].

Table 1. Hypothalamic norepinephrine levels following drug administration*

Group	N	NE (ng/g ± S.E.)	% Change
Saline	21	1905 ± 35	
Reserpine placebo + pridefine (30 min)	11	2316 ± 59†‡	+22
Reserpine placebo + pridefine (60 min)	10	1987 ± 53	+4
Reserpine	19	126 ± 6	
Reserpine + pridefine (30 min)	9	200 ± 18§‖	+59
Reserpine + pridefine (60 min)	10	129 ± 8	+2

* Pridefine was given i.p. at 25 mg/kg. Values were pooled from two experiments; comparable results were obtained with independent analyses.

† Differs significantly from saline group, $P < 0.001$.

‡ Differs significantly from reserpine placebo + pridefine group (60 min), $P < 0.001$.

§Differs significantly from reserpine group, $P < 0.001$.

‖ Differs significantly from reserpine + pridefine group (60 min), $P < 0.001$.

Pridefine had a small effect on DA levels in the striatum. Both control and reserpinized rats showed a slight increase in DA 60 min after pridefine (12 and 14 per cent, respectively), but these increases are not statistically significant. No significant alterations in DA levels were observed in either control or pretreated rats in the hypothalamus, frontal cortex or nucleus accumbens at either time. Control values for each region were comparable to those obtained elsewhere [19–21].

Levels of 5-HT were not altered significantly at either time in the hypothalamus, frontal cortex or striatum of unpretreated or reserpinized rats. However, a 28 per cent increase in hypothalamic 5-HT was observed in reserpinized rats 30 min post-injection but this increase also is not statistically significant. Control values for 5-HT in hypothalamus were similar to those reported elsewhere [7]. Serotonin levels measured in the striatum were slightly lower (303 ng/g vs 418 ng/g) than those obtained by Kelley *et al.* [21]; this difference is probably due to the more specific electrochemical detection of 5-HT employed in the present study.

In vitro *uptake.* Pridefine is an effective uptake inhibitor of tritiated NE, DA and 5-HT. To determine whether a particular monoamine and/or brain region is maximally or preferentially affected by the drug, IC_{50} values were calculated for all three transmitters. The results, ranked in order of potency, are presented in Table 2. NE uptake was most potently inhibited in the hypothalamus ($IC_{50} = 2.4 \times 10^{-6}$ M). Although the IC_{50} for NE in striatum was identical (2.4×10^{-6} M), this result must be viewed with caution. Cooper *et al.* [22] have reported only 250 ng/g NE in the striatum as compared to 7500 ng/g DA. It is possible that the affinity of dopaminergic nerve terminals for tritiated NE is sufficiently high to yield this result. Dopamine uptake was most potently inhibited in the midbrain ($IC_{50} = 8.9 \times 10^{-7}$ M). The hypothalamus was the site of the most potent inhibition of 5-HT ($IC_{50} = 1.3 \times 10^{-6}$ M).

Ex vivo *uptake.* To determine whether the results obtained in *in vitro* uptake experiments have physiological significance, the uptake studies were repeated with synaptosomal preparations from whole brain of rats pretreated with pridefine (50 mg/kg, i.p.). The per cent inhibition measured for each transmitter at various times of decapitation are presented in Table 3. These percentages reflect the total amount of tritiated transmitter taken up in the presence of drug since no accurate corrections for diffusion and binding could be performed in these preparations. Pridefine had a maximal effect on NE uptake at 30 min, with 18.5 per cent inhibition. This degree of inhibition differed significantly ($P < 0.05$) from inhibition at all times except 60 min. At 60 min, the per cent inhibition dropped to about one-half that measured at 30 min, indicating that the drug effect lasted for about 1 hr. Inhibition of DA uptake was maximal at 90 min ($P < 0.05$), but inhibition did not differ significantly at either 60 or 120 min (14.1 and 13.4 per cent, respectively). Thus, it appears that the drug effect on DA uptake increases to a maximum by 90 min and is sustained for at least 2 hr. Similarly, uptake inhibition of 5-HT peaked at 60 min ($P < 0.05$) and declined gradually through 120 min; nevertheless, inhibition was still significant (22 per cent) 2 hr after drug administration. Since no other time points were examined, it must be concluded that the drug effect on 5-HT uptake was sustained for at least 2 hr. Thus, a single dose of pridefine significantly inhibits the *ex vivo* uptake of all three neurotransmitters; the duration of the effect was greatest in the uptake of 5-HT.

Table 2. IC_{50} Values of ^{3}H-amine uptake by rat brain synaptosomes*

Transmitter	Tissue	IC_{50} (M)
NE†	Hypothalamus	2.4×10^{-6}
	Striatum	2.4×10^{-6}
	Hindbrain	8.1×10^{-6}
	Midbrain	1.7×10^{-6}
	Frontal cortex	1.1×10^{-6}
DA‡	Midbrain	8.9×10^{-7}
	Frontal cortex	1.6×10^{-6}
	Hindbrain	2.0×10^{-6}
	Hypothalamus	2.5×10^{-6}
	Striatum	2.8×10^{-6}
5-HT§	Hypothalamus	1.3×10^{-6}
	Striatum	2.6×10^{-6}
	Hindbrain	5.4×10^{-6}
	Midbrain	6.9×10^{-6}
	Frontal cortex	1.0×10^{-5}

* The IC_{50} values (the concentration of drug that inhibited uptake by 50 per cent) were calculated by log–logit analysis.

† Control values for uptake expressed as pmoles·8 min^{-1}·mg protein^{-1} (n = 6): frontal cortex, 7.19 ± 0.102; striatum, 40.56 ± 0.645; hypothalamus, 12.41 ± 0.064; midbrain, 7.34 ± 0.101; and hindbrain, 8.42 ± 0.077.

‡ Control values for uptake expressed as pmoles·8 min^{-1}·mg protein^{-1} (n = 6): frontal cortex, 4.00 ± 0.036; striatum, 57.94 ± 1.66; hypothalamus, 8.86 ± 0.150; midbrain, 4.40 ± 0.057; and hindbrain, 4.65 ± 0.076.

§ Control values for uptake expressed as pmoles·8 min^{-1}·mg protein^{-1} (n = 6): frontal cortex, 9.82 ± 0.065; striatum, 11.50 ± 0.183; hypothalamus, 14.28 ± 0.195; midbrain, 11.02 ± 0.153; and hindbrain, 9.24 ± 0.131.

Table 3. *Ex vivo* uptake inhibition in whole brain*

Time of decapitation (min)	NE % Inhibition	DA % Inhibition	5-HT % Inhibition
15	2.9	8.3	16.5†
30	18.5†‡§	8.5	24.8†‡
60	9.3	14.1	30.4†‡§
90	3.9	16.5†	23.5†‡
120	−3.5	13.4	21.7†‖

* Control values for uptake expressed as pmoles·8 min^{-1}·mg protein^{-1} (mean ± S.E.M., n = 6); NE, 8.4 ± 0.399; DA, 13 ± 0.766; and 5-HT, 10.2 ± 0.512.

† Differs significantly from control, $P < 0.05$.

‡ Differs significantly from 15 min, $P < 0.05$.

§ Differs significantly from 90 min, $P < 0.05$.

‖ Differs significantly from 60 min, $P < 0.05$.

Table 4. RC_{50} Values of *in vitro* ^{3}H-amine release by rat brain synaptosomes*

Transmitter	Tissue	RC_{50} (M)
NE	Whole brain	2.7×10^{-6}
	Striatum	4.1×10^{-5}
	Hindbrain	8.6×10^{-5}
	Hypothalamus	9.2×10^{-5}
	Midbrain	9.8×10^{-5}
	Frontal cortex	1.3×10^{-4}
DA	Striatum	4.3×10^{-6}
	Hindbrain	2.2×10^{-5}
	Hypothalamus	2.5×10^{-5}
	Whole brain	2.9×10^{-5}
	Midbrain	3.0×10^{-5}
	Frontal cortex	3.9×10^{-5}
5-HT	Striatum	3.9×10^{-5}
	Frontal cortex	4.2×10^{-5}
	Midbrain	5.3×10^{-5}
	Hindbrain	5.4×10^{-5}
	Hypothalamus	5.5×10^{-5}
	Whole brain	2.3×10^{-4}

* The RC_{50} values (the concentration of drug that enhanced the release of ^{3}H-amines by 50 per cent) were calculated by log–logit analysis.

Release experiments. In preliminary experiments, the efficacy of the drug as a releasing agent was assessed with synaptosomal preparations from whole brain. At the lowest concentration tested (10^{-6}M), the drug was most effective in enhancing the release of NE (36 per cent) compared to DA (9 per cent) and 5-HT (10 per cent). Subsequent analyses were performed in specific regions. Overall, the drug was more efficacious in blocking uptake than in enhancing release of the tested neurotransmitters. The RC_{50} values (drug concentration that produces 50 per cent transmitter release), ranked in order of potency, are presented in Table 4. NE release was most pronounced in whole brain ($RC_{50} = 2 \times 10^{-6}$M). DA release was enhanced predominantly in the striatum ($RC_{50} = 4 \times 10^{-6}$M). The lowest RC_{50} for 5-HT was also observed in the striatum.

DISCUSSION

The present study was designed to evaluate certain biochemical pharmacological properties of the novel antidepressant pridefine. Initial studies revealed that a single dose of pridefine results in time-dependent increases in amine levels. The drug inhibited synaptosomal uptake of NE, DA and 5-HT *in vitro* and *ex vivo* but also enhanced the release of these transmitters. To determine which effect predominates, the criterion established by Heikkila *et al.* [6] was employed. Using dose–response curves, these authors have shown that a drug has a mixed mode of action if plots of the per cent inhibition overlap with plots of the per cent release at identical drug concentrations. In addition to dose–response curves (Fig. 2), analogous comparisons were made using IC_{50} and RC_{50} values. Identical effective concentrations indicated that the drug has a mixed mode of action, whereas a 10-fold or more concentration difference indicated that the mechanism affected by the lower drug concentration predominates. For each transmitter, some degree of correlation between the effect of pridefine on amine levels and the ability of the drug to block *ex vivo* uptake was observed.

The most striking effects were observed with NE. In all brain regions examined, the RC_{50} was at least ten times greater than the corresponding IC_{50}, illustrating the predominance of uptake blockade. A comparison of the IC_{50} calculated for pridefine in the hypothalamus (2.4×10^{-6}M) with those calculated for the tricyclic antidepressants reveals that pridefine is closest in potency to imipramine (10^{-6}M) [23]. These results parallel the clinical studies indicating

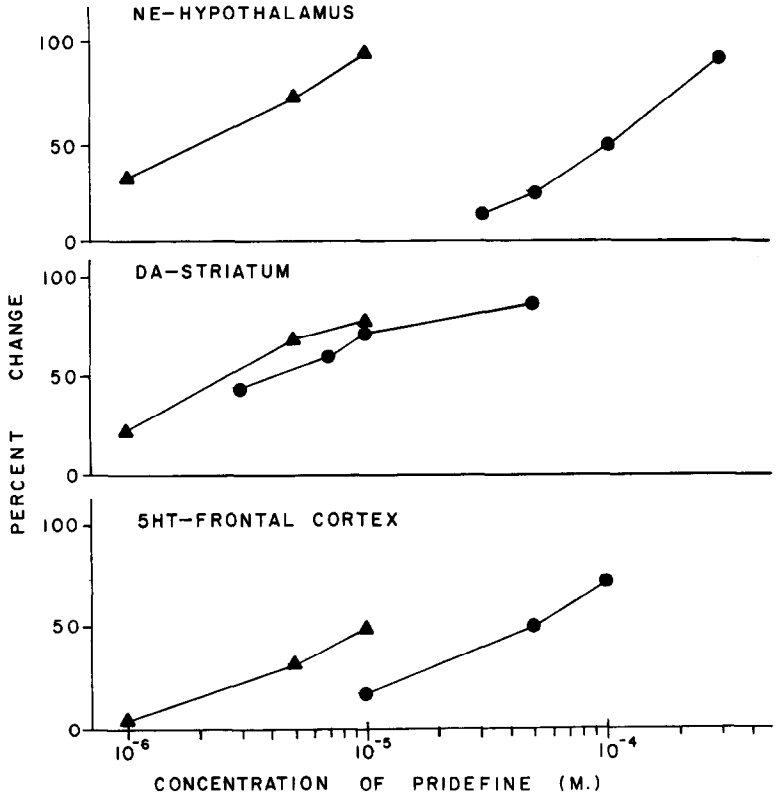

Fig. 2. Dose–response curves illustrating the effects of various concentrations of pridefine on ^{3}H-amine uptake and release. The per cent change shown is the mean ± S.E. of quadruplicate determinations. The predominance of [^{3}H]NE uptake blockade in the hypothalamus is evident. Here the dose–response curve for uptake is far to the left of that representing the effect of the drug on release. Overlapping dose–response curves demonstrate a mixed mode of action for DA in striatum. The mode of action is not evident for 5-HT in frontal cortex. However, data presented in Tables 2 and 4 indicate that pridefine has a mixed mode of action for 5-HT in frontal cortex. Key: (▲——▲) ^{3}H-amine uptake; and (●——●) ^{3}H-amine release.

that pridefine is at least as effective as imipramine [2, 3]. There is a good correlation between *ex vivo* uptake inhibition and the effect of pridefine on hypothalamic levels of NE. The increase in hypothalamic NE at 30 min following drug in both control and reserpinized rats parallels the maximal uptake inhibition observed in whole brain also 30 min after drug. It is possible that reuptake accounts for the increase in NE. A similar explanation had been postulated for 5-HT increases observed after chlorimipramine [4]. Because tricyclic antidepressants have only weak inhibitory activity on MAO [4] and because pridefine does not inhibit MAO, the increase in hypothalamic levels of NE is probably related to reuptake blockade. It is also noteworthy that plasma levels of the NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) are altered in a time-dependent manner during treatment with pridefine [3]. That study also showed a significant correlation between changes in MHPG levels and the rate of clinical improvement in a population of depressed patients [3].

A mixed mode of action is indicated for DA in the striatum with an IC_{50} of 2.8×10^{-6} M and an RC_{50} of 4.4×10^{-6} M. A similar observation was made when striatal uptake and release were examined in the presence of tricyclic antidepressants [6]. For all other regions, inhibition of DA uptake predominated. Some correlation between *ex vivo* uptake inhibition and the effect of pridefine on DA levels is apparent. Minor increases in striatal DA were observed 60 min post-drug for control and reserpinized rats (12 and 14 per cent, respectively). Uptake inhibition, however, was evident at 60 min and reached a maximum at 90 min. Had levels been measured beyond 60 min, it is possible that statistically significant increases would have been observed for both control and reserpinized rats.

Inhibition of 5-HT uptake was pronounced in all regions except frontal cortex. In hypothalamus and striatum, the RC_{50} was at least ten times greater than the corresponding IC_{50}. In frontal cortex, however, the RC_{50} and IC_{50} were nearly identical, implying a mixed mode of action of the drug in this region. Although the *ex vivo* uptake of 5-HT was inhibited to a greater degree than that of NE or DA (24.8 per cent at 30 min and 30.4 per cent at 60 min) and for a longer duration (inhibition remained well above control through 120 min), the increase in 5-HT levels did not approach the magnitude observed for NE. Although an IC_{50} of 2.0×10^{-6} M was observed for 5-HT in the hypothalamus, hypothalamic 5-HT levels were not significantly altered by the drug. The only change observed in 5-HT levels occurred in the frontal cortex where pridefine is least effective in blocking 5-HT uptake. Control levels did not change at either time point, but a nonsignificant increase of 28 per cent was observed in reserpinized rats 30 min after injection.

In summary, the pyrrolidine derivative pridefine is a clinically effective, novel antidepressant which structurally does not resemble any of the commercially available compounds. The drug is predominantly a reuptake blocker, even though it possesses amine releasing activity. Uptake inhibition is comparable among the three transmitters studied and no specific brain region stands out in inhibitory or releasing potency of the drug. With respect to regional amine levels, the most consistent and pronounced effects were obtained in levels of hypothalamic NE. The action of the drug in inhibiting amine uptake was confirmed in *ex vivo* experiments, indicating that the compound reaches active sites in the brain following peripheral administration. The type of uptake inhibition that this drug produces is currently under investigation and will be discussed in a separate publication.

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