clearly indicate that DU-717 shows a dose-related and sustained reduction in blood pressure without any change in urine volume, electrolyte excretion and carbohydrate metabolism. Subacute toxicity of DU-717 was examined in male Sprague-Dawley rats. At a dose of 1000 mg/kg p.o. for 30 days, DU-717 caused no significant change in the weight gain and organ weight or no appreciable effect on the hematobiochemical findings as compared with the control. The general hemodynamic actions of DU-717, and the mechanisms responsible for its hypotensive effect, are currently under investigation.

It may be concluded that DU-717 is a compound having a novel profile, different from those of the antihypertensive drugs of benzothiadiazine diuretics or diazoxide in its pharmacological properties and underlying mode of action. It seems, therefore, worthwhile to study the clinical efficacy of this compound in the treatment of patients with essential hypertension.

Effects of 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines and related compounds, potential antidepressants, on biogenic amine uptake mechanisms

T. A. Pugsley and W. Lippmann[1]

Biochemical Pharmacology Department, Ayerst Research Laboratories, Box 6115, Montreal (Quebec, Canada H3C 3J1), 30 April 1976

Summary. A new series of compounds structurally related to the potential antidepressant tandamine, i.e., the 1-alkyl-1,2,3,4-tetrahydrocarbazole-1-ethanamines, inhibit the noradrenaline uptake mechanism and are relatively ineffective in inhibiting the serotonin uptake mechanism in vivo. The potency of the most effective compound (9-ethyl-N,N,1-trimethyl-1,2,3,4-tetrahydrocarbazole) is similar to that of desimipramine and is of potential use in the treatment of endogenously depressed patients.

Recent studies[2–5] demonstrate that a new potential antidepressant drug tandamine hydrochloride (9-ethyl-*N, N,* 1-trimethyl-1, 2, 3, 4-tetrahydrothiopyrano [3, 4-b] indole-1-ethanamine hydrochloride) (Table 1) possesses both a biochemical and pharmacological profile in animals qualitatively similar to, but lacking the central anticholinergic activity of, the tricyclic antidepressant desimipramine (DMI). Tandamine is greater, or equivalent, in potency to DMI as an inhibitor of noradrenaline (NA) reuptake mechanism and, like DMI, is relatively ineffective in inhibiting the 5-HT uptake mechanism[2–4]. In the present studies a series of compounds, structurally related to tandamine (Table 1), i.e., the 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines and related compounds (cycloalkanoindoles), have been examined for their inhibitory activities on NA and 5-HT reuptake mechanisms; there is considerable evidence that the antidepressive action of certain known tricyclic antidepressants may be due to their abilities to cause inhibition of the neuronal reuptake of NA[6], 5-HT[7], or both.

Materials and methods. The cycloalkanoindoles were synthesized by Drs A. Asselin and L. G. Humber and Mrs J. Komlossy, Ayerst Research Laboratories[8]. Desimipramine hydrochloride (Pertofrane) and imipramine hydrochloride (Tofranil) (I) were gifts from Ciba-Geigy.

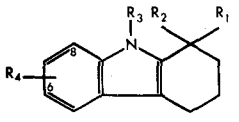
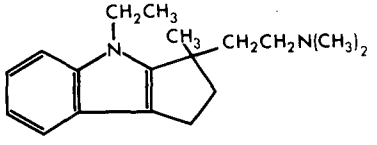
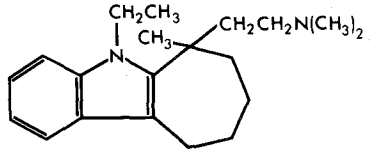
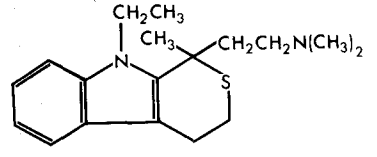
The determination of the effects of the compounds on ^{3}H-NA uptake and release in the hearts of albino mice (23–25 g, Canadian Breeding Laboratories) was carried out as previously described[3, 9] as was their ability to potentiate the 5-hydroxytryptophan (5-HTP)-induced behavioural syndrome in mice[7]. The determination of the effects of the test compound on the α-4-dimethyl-3-hydroxy-phenylethylamine (H77/77)-induced depletion of brain NA and dopamine (DA) was carried out[11] and the concentrations of brain NA and DA were determined[12–14]. Percent inhibition exhibited by the test compounds of the H77/77-induced decline in brain NA was calculated by utilizing the formula employed by Bruinvels[15].

Results and discussion. Compounds *1, 2, 3, 5, 6, 8, 9* and *10,* and also DMI and I, when given before the ^{3}H-NA, decreased the ^{3}H-NA concentrations in mouse heart displaying ED_{50}'s < 10 mg/kg, i.p. (Table 1). None of these compounds when administered at 10 mg/kg, i.p., after the ^{3}H-NA altered the ^{3}H-NA as compared to controls [e.g., controls: 48,630 ± 1784; *2*: 53,137 ± 1167; I: 53,810 ± 2338 (cpm/g ± S.E.)]. Thus, the cycloalkanoindoles, like DMI and I, blocked the uptake and did not cause an increased release of ^{3}H-NA. It is apparent that the presence of an ethyl group on the indole nitrogen, a methyl group and a dimethylaminoethyl side chain at position 1 in the 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines, i.e., compound *2*, results in maximal activity (ED_{50}: 0.7 mg/kg, i.p.). Lengthening the chain on the ring nitrogen to the propyl (*5*) or shortening the length to a methyl (*1*) decreased the activity. Reduced activity was also caused by replacement of the dimethylamino group of the side chain by a methylamino (*3*). The presence of an ethyl group at position 8 (*7*) caused a decrease in activity and a chloro group at position 6 (*8*) also re-

[1] The authors acknowledge the technical assistance of Mrs J. Farnsworth, Mrs B. Gut and Mr J. Lacasse.

[2] W. Lippmann and T. A. Pugsley, Pharmacologist *17*, 258 (1975).
[3] W. Lippmann and T. A. Pugsley, Biochem. Pharmac. *25*, 1179 (1976).
[4] T. A. Pugsley and W. Lippmann, Psychopharmacologia *47*, 33 (1976).
[5] I. Jirkovsky, L. G. Humber, K. Voith, M.-P. Charest, T. A. Pugsley and W. Lippmann, 169th ACS National Meeting, Philadelphia, Pa., April 1975, Abstr. Med. 34.
[6] J. Glowinski and J. Axelrod, Pharmac. Rev. *18*, 775 (1966).
[7] A. Carlsson, J. Jonason, M. Lindqvist and K. Fuxe, Brain Res. *12*, 456 (1969).
[8] A. A. Asselin, L. G. Humber, J. Komlossy and M.-P. Charest, J. Med. Chem. (in press).
[9] W. Lippmann, J. Med. *2*, 250 (1971).
[10] A. Carlsson, H. Corrodi, K. Fuxe and T. Hökfelt, Eur. J. Pharmac. *5*, 357 (1969).
[11] A. Carlsson, H. Corrodi, K. Fuxe and T. Hökfelt, Eur. J. Pharmac. *5*, 367 (1969).
[12] L. G. Whitby, J. Axelrod and H. Weil-Malherbe, J. Pharmac. exp. Ther. *132*, 193 (1961).
[13] U. S. Von Euler and I. Floding, Acta Physiol. scand. *33*, Suppl. *118*, 45–47 (1955).
[14] R. Laverty and K. M. Taylor, Anal. Biochem. *22*, 269 (1968).
[15] J. Bruinvels, Br. J. Pharmac. *42*, 281 (1971).

Table 1. Inhibition o the uptake of ^{3}H-noradrenaline (^{3}H-NA) and potentiation of the 5-hydroxytryptophan (5-HTP)-induced syndrome

Compound						Inhibition of ^{3}H-NA uptake ED_{50}: (mg/kg, i.p.)	Potentiation of 5-HTP Syndrome score (dose: 25 mg/kg, i.p.)
	R_1	R_2	R_3	R_4	x		
1	$CH_2CH_2N(CH_3)_2$	CH_3	CH_3		HCl	2.2	+1
2	$CH_2CH_2N(CH_3)_2$	CH_3	CH_2CH_3		HCl	0.7	+1
3	$CH_2CH_2NHCH_3$	CH_3	CH_2CH_3		HCl	6.8	+1
4	$CH_2CH_2N(CH_3)_2$	H	CH_3		HCl	>10	+2
5	$CH_2CH_2N(CH_3)_2$	CH_3	$CH_2CH_2CH_3$		HCl	1.6	+1
6	$CH_2CH_2N(CH_3)_2$	CH_2CH_3	CH_2CH_3		HBr	2.1	+2
7	$CH_2CH_2N(CH_3)_2$	CH_3	CH_2CH_3	8-CH_2CH_3	maleate	>10	+1
8	$CH_2CH_2N(CH_3)_2$	CH_3	CH_2CH_3	6-Cl	HCl	4.5	+2
9					HBr	4.7	I
10					HCl	1.8	I
Imipramine					HCl	6.0	+3
Desimipramine					HCl	1.0	+1
(Tandamine					HCl	0.3	+1)

For ^{3}H-NA uptake, test compounds were injected 45 min before or after the ^{3}H-NA; the animals (8 per group) were killed 2 h after the administration of the test compounds and the concentrations of ^{3}H-NA then determined.
For 5-HTP potentiation, the test compounds (25 mg/kg, i.p.) were administered to mice (5 per group) 30 min before 5-HTP (300 mg/kg, i.p.) and the syndrome (extension and abduction of hindlimbs, tremors, lordosis, head twitching and excitation) scored as ineffective (I), +1 (weak effect) to +4 (very strong effect).

Table 2. Effects on the H77/77-induced depletion of brain noradrenaline (NA)

Treatment	Dose (mg/kg, i.p.)	Compound alone	Compound + H77/77	Percent inhibition	ED_{50} (mg/kg, i.p.)
Saline	–	100.0 ± 5.2	60.9 ± 5.5[a]		
Compound *2*	12.5 + 6.3	88.7 ± 1.6	84.6 ± 4.6[b]	88	6.3
	6.3 + 3.1	99.5 ± 5.8	77.3 ± 1.5[c]	43	
	3.1 + 1.6	100.8 ± 1.9	74.1 ± 4.8	32	
Saline	–	100.0 ± 2.6	64.7 ± 2.1[a]		
Desimipramine	12.5 + 6.3	94.6 ± 2.4	83.0 ± 1.8[b]	65	9.6
	6.3 + 3.1	100.3 ± 2.0	76.4 ± 4.6[c]	32	
	3.1 + 1.6	100.8 ± 5.7	71.4 ± 2.8	17	

H77/77 (12.5 mg/kg, i.p.) was injected at 0 and 2 h and the mice killed 2 h later after the last dose. The test compound was injected 30 min prior to H77/77, the second dose being half the first.
Each value is mean of 4 or 5 determinations and is expressed as percent of concentrations (μg/g ± S.E.) of NA (0.29 ± 0.01) in saline-treated animals.
[a] $p < 0.001$ vs saline-treated animal; [b] $p < 0.01$; [c] $p < 0.05$ vs H77/77-treated animals.

sulted in loss of activity. The significance of the methyl group at position 1 was indicated by the reduced activity of the compound containing an ethyl group (*6*). This was also shown when the group on the ring nitrogen was a methyl (*7*) and the methyl group at position 1 was replaced by a hydrogen atom, the resulting compound (*4*) being ineffective. In comparison with the tetrahydrocarbazole compound *2*, the cyclopentano indole (*9*) and cycloheptano indole (*10*) exhibited decreased activities of 6- and 2-fold, respectively. The same structural features for maximal inhibition of ^{3}H-NA uptake have been shown for the respective compound in the 1-aminoalkyl-1, 3, 4, 9-tetrahydrothiopyrano [3, 4-b] indole series (tandamine, ED_{50}: 0.3 mg/kg, i.p.) and 1-aminoalkyl-1, 3, 4, 9-tetrahydropyrano [3, 4-b] indole series (1, 9-dimethyl-1-[2-(dimethylamino)ethyl]-1, 3, 4, 9-tetrahydropyrano [3, 4-b] indole hydrochloride, ED_{50}: 1.5 mg/kg, i.p.)[3]. Potent antagonism of reserpine-induced ptosis has been demonstrated for compound *2* with varying activities for its analogs[8]. In this regard, the potent inhibition of the NA uptake by compound *2* and varying activities of the analogs, could be the mechanism of action of these compounds.

H77/77 is a depletor of brain catecholamines and utilizes the neuronal uptake mechanism to bring about this depletion with its action on NA depletion being blocked by compounds which are known NA uptake inhibitors[11]. Thus, inhibition by compounds of the H77/77-induced depletion of NA and DA gives a measure of inhibition of neuronal NA or DA uptake. The H77/77-induced depletion of NA was blocked by compound *2*, which exhibited a potency equivalent to that of DMI (Table 2) thus indicating that compound *2* blocks NA uptake centrally in addition to that in the periphery. Neither compound *2* nor DMI[11] alone affected endogenous NA (Table 2); endogenous DA and the H77/77-induced depletion of DA were not affected by compound *2* or DMI (not shown) indicating a specificity in action.

Potentiation of the 5-HTP-induced behavioural effects has been shown to generally correlate with the ability of compounds to inhibit the brain uptake of 5-HT[3, 7, 10], provided that the compounds are not inhibitors of monoamine oxidase or releasers of 5-HT[7, 10]. Thus, activity in this model is an indication of the ability of compounds to affect 5-HT-related mechanisms. I caused a dose-related potentiation of the 5-HTP syndrome at 25 mg/kg, i.p. (+3, Table 1), 12.5 mg/kg, i.p. (+2) and 6.25 mg/kg, i.p. (+1). None of the cycloalkanoindoles examined potentiated the 5-HTP-induced activity to at least the level of I, a known blocker of 5-HT uptake[9], and thus were considered to be relatively ineffective. Compounds *4, 6 and 8 did exhibit an activity of +2*; compound *2* showed only a +1 activity. Compound *2* was thus similar to DMI since this latter drug did not exhibit appreciable activity in the present and previously reported[2–4] studies.

The present study reveals a new series of compounds structurally related to tandamine, i.e., the 1-alkyl-1, 2, 3, 4-tetrahydrocarbazole-1-ethanamines, which inhibit the NA uptake mechanism, the most potent compound (*2*) being equivalent to DMI and lacking an appreciable ability to affect 5-HT mechanisms thus being a relatively specific NA uptake blocker. Clinically, compound *2* possessing such a profile would be expected to be of potential use in treating endogenously depressed patients, particularly those with a decreased drive[10, 11].

Effect of milk on plasma unbound-bilirubin concentration in homozygous Gunn rat sucklings

H. Sato and R. Semba

Department of Perinatology, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480–03 (Japan), 23 February 1976

Summary. Sucking of milk increased plasma unbound-bilirubin concentration in the homozygous Gunn's strain of jaundiced rats. Administration of skim milk did not increase unbound-bilirubin, while a mixture of fatty acids and skim milk elevated it.

Most bilirubin is bound to albumin in the plasma. The albumin-bound bilirubin cannot penetrate tissues, while the dissociated bilirubin, called 'unbound-bilirubin', may do so and thereby injure the cells[1–3]. Thus, plasma unbound-bilirubin concentration is closely correlated to the risk of kernicterus[4–7]. In the present study, effect of milk on unbound-bilirubin concentration was investigated to ascertain whether milk could displace bilirubin from albumin in the homozygous Gunn's strain of jaundiced rats.

Materials and methods. Animals. Gunn's strain of jaundiced rats (j/j; 14-day-old) were used throughout the study. Littermates diagnosed by yellow skin colour as j/j were equally allotted to experimental and control groups. Animals were maintained on laboratory diet (NMF, Oriental Yeast Co.) and tap water.

Starvation experiments. Experimental rats were isolated from their mothers and placed in an incubator at 30 °C from 5.00 p.m. till 9.00 a.m. of the next day (16 h). Physiological saline solution was administered orally at 9.00 a.m. to compensate exactly for loss of body weight during the period. Controls were left with their mothers and nursed ad libitum through the same period. Actual sucking of milk was verified by examination of the shape and weight of stomach. Blood was sampled at 10.00 a.m.

Milk administration experiments. After starvation for 16 h as described above, experimental rats were given orally 0.8 ml each of mother's milk curd solution (a 2 g wet weight of milk curds recovered from the stomach of other sucking rats was dissolved in 1 ml of 0.1 M Na_2CO_3) at 9.00 a.m. and 11.00 a.m. The controls received physiological saline instead of milk curd solution. Blood was sampled at 3.00 p.m. Human breast milk, bovine powdered milk (1 g Meiji FM-U soft curd milk dissolved in

1 R. Zetterström and L. Ernster, Nature *178*, 1335 (1956).
2 R. Kato, S. Kashiwamata and F. Niwa, Brain Res. *83*, 81 (1975).
3 M. L. Cowger, R. P. Igo and R. F. Rabbe, Biochemistry *4*, 2765 (1965).
4 G. B. Odell, J. Pediat. *55*, 268 (1959).
5 I. Diamond and R. J. Schmid, J. Clin. Invest. *45*, 678 (1966).
6 P. Zamet, H. Nakamura, S. Perez-Robles, J. C. Larroche and A. Minkowski, Biol. Neonat. *26*, 274 (1975).
7 H. Sato and H. Yamamura, K. Ohta and M. Sato, Teratology *10*, 96 (1974).