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THE EFFECTS OF TANDAMINE, A NEW POTENTIAL ANTIDEPRESSANT AGENT, ON BIOGENIC AMINE UPTAKE MECHANISMS AND RELATED ACTIVITIES

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Abstract — The effects of various thiopyrano [3,4-b]indoles and pyrano [3,4-b] indoles on norepinephrine (NE) and 5-hydroxytryptamine (5-HT) uptake were determined in mice. A thiopyranoindole, tandamine,* was a potent inhibitor of [^{3}H]NE uptake in the heart, being three times more active than desimipramine, and was relatively ineffective in potentiating the 5-hydroxytryptophan (5-HTP) behavioural syndrome. A pyranoindole and a thiopyranoindole blocked both [^{3}H]NE and brain 5-HT uptake with activities greater than, or similar to imipramine. Structure–activity relationships for these two activities were determined. Tandamine was the most potent in antagonizing reserpine-induced hypothermia and the guanethidine-induced depletion of heart [^{3}H]NE. The (−)enantiomer of tandamine exhibited greater activity in blocking NE and 5-HT uptake. The results indicate that tandamine and certain of its congeners, differing chemically from the known tricyclic antidepressants, exert relatively selective stereochemical effects on NE and 5-HT uptake mechanisms. Such agents are potentially useful as antidepressants and as tools for further studies of the uptake mechanism and functional significance of NE and 5-HT.

The principal mechanism for the inactivation of injected or endogenously released norepinephrine (NE) is re-uptake into adrenergic neurons by an active neuronal membrane transport mechanism [1, 2], usually designated as the membrane pump. Such active transport mechanisms have been shown in both peripheral and central catecholamine neurons [3, 4]. A similar active transport mechanism has been shown to exist in central dopamine (DA) and 5-hydroxytryptamine (5-HT) neurons [4]. Blockade of the monoamine membrane pumps by certain tricyclic antidepressant drugs results in a decrease in the inactivation of the monoamines and a subsequent potentiation of their effects both in the periphery and the central nervous system [4–6].

Various tricyclic antidepressants have been shown to exert some specificity in blocking the monoamine pumps [7, 8]. The secondary amines, e.g. desimipramine and protriptyline, are more potent blockers of the membrane pump in central NE neurons than the tertiary amines, e.g. chlorimipramine, imipramine and amitriptyline. The converse is true for blockade of the membrane pump of central 5-HT neurons. None of the drugs had any significant effect on the membrane pump of central DA neurons.

In the present studies members of two series of compounds, i.e. the pyrano [3, 4-b] and thiopyrano [3, 4-b]indoles, have been found to exhibit blocking activities with regard to the NE and 5-HT membrane pumps. One of these agents, tandamine, is shown to be relatively selective in blocking NE uptake and is currently of interest clinically.

MATERIALS AND METHODS

Radioactive norepinephrine levels. Male albino mice, (23–25 g; Canadian Breeding Laboratories) were injected in the tail vein with 0.25 ml containing 2.5 μCi *dl*-[7-^{3}H]norepinephrine–HCl (7.7 Ci m-mole; Radiochemical Centre, Amersham), ([^{3}H]NE), in a solution of 0.75% NaCl and 0.01 N HCl. Test compounds were injected intraperitoneally (i.p.) in 0.5 ml 0.025% acetic acid; the doses refer to the salt of the compound. The tissue samples were homogenized in ice-cold 0.4 N perchloric acid and centrifuged. A portion of the supernatant fluid was transferred to a vial containing a mixture of 1 ml methanol, 3 ml ethanol and 10 ml toluene-phosphor [0.4% 2,5-diphenyloxazole and 0.005% 1,4-bis (5-phenyl-oxazole-2-yl) benzene], and the total radioactivity was measured by liquid scintillation counting (efficiency, 10 per cent). The radioactivity in the heart at times comparable to those of the present studies is almost entirely due to [^{3}H]NE [9].

5-Hydroxytryptophan-induced syndrome. The behavioural syndrome produced by potentiation of the effects of *dl*-5-hydroxytryptophan (5-HTP) consists of head twitches, lordosis, tremors, extension and abduction of hind limbs and excitation [10, 11]. Male albino mice (23–25 g) were administered the test compounds (i.p.) 30 min prior to the administration of 5-HTP (300 mg/kg, i.p.) and the syndrome was scored after 15 min. The degree of intensity of the 5-HTP syndrome following the compounds is indicated by an arbitrary scale: ineffective (I); +1 (weak effect); +2 (moderate effect); +3 (strong effect); +4 (very strong effect). Any changes in gross behaviour prior to the injection of 5-HTP were also noted. 5-HTP alone did not cause any changes under these conditions.

Antagonism of reserpine-induced hypothermia. Groups of ten male albino mice (23–25 g) were pretreated (i.p.) with graded doses of the test agents 1 hr before administration of reserpine (1 mg/kg, s.c.). One group was kept as control (saline) and another group received saline plus reserpine. Rectal temperature was measured with a YSI telethermometer and

*1-[2-(dimethylamino)ethyl]-9-ethyl-1,3,4,9-tetrahydro-1-methyl-thiopyrano [3,4-6]indole hydrochloride.

402 physiological probe before drug treatment and every hour after the treatment for 5 hr.

Antagonism of the displacement of [^{3}H]NE by guanethidine. Male albino mice (23–25 g) were injected in the tail vein with 2.5 μCi of [^{3}H]NE. Test agents were given i.p. 45 min after, and guanethidine sulphate (20 mg/kg, i.p.) 75 min after the [^{3}H]NE administration. All animals were killed 4 hr after the [^{3}H]NE injection, the hearts removed and total radioactivity determined as described previously.

As some of the test agents, when given alone, decreased the spontaneous release of the [^{3}H]NE from mouse heart, the per cent displacement of [^{3}H]NE by guanethidine was calculated utilizing a formula similar to that proposed by Bruinvels [12]:

$$\text{Per cent inhibition} = \left[\frac{\left(\frac{[^3\text{H}]\text{NE agent + guan.} \quad [^3\text{H}]\text{NE guan.}}{[^3\text{H}]\text{NE agent} \quad [^3\text{H}]\text{NE saline}} \right)}{\left| \frac{[^3\text{H}]\text{NE guan.}}{[^3\text{H}]\text{NE saline}} \right|} \right] \cdot 100$$

Antagonism of displacement of 5-HT induced by α-ethyl-3-hydroxy-4-methyl phenylethylamine (H75/12). Male albino mice (23–25 g) were injected with two doses of H75/12 (25 mg/kg, i.p.) 2 hr apart. All animals were killed 2 hr after the last dose of H75/12. The test agents were injected 30 min before the H75/12, the second dose of the agents being half the first.

Mice were killed, whole brains quickly removed, rinsed in saline and frozen on dry ice. 5-HT was extracted [13] and assayed spectrofluorimetrically [14]. Per cent inhibition of the displacement of 5-HT by H75/12 for each of the agents was calculated by the formula used above for the guanethidine-induced displacement of [^{3}H]NE, i.e. by substituting 5-HT for [^{3}H]NE and H75/12 for guan.

In both of the experimental models, i.e. uptake of [^{3}H]NE and guanethidine-induced depletion of [^{3}H]NE, the per cent inhibition of activity vs the log dose (mg/kg) was plotted and the dose at which 50 per cent inhibition occurred (ED_{50}) was read from graph.

The agents employed in the studies were gifts from Ciba-Geigy Ltd. guanethidine sulphate (Ismelin); reserpine injection U.S.P. (Serpasil); imipramine (Tofranil) and desimipramine (Pertofrane) hydrochlorides and Merck, Sharp & Dohme Ltd. amitriptyline hydrochloride (Elavil). Butriptyline hydrochloride (Evadyne) was from Ayerst Laboratories. Nortriptyline hydrochloride was prepared by Dr. M. A. Davis, Chemistry Department, Ayerst Laboratories. *dl*-5-Hydroxytryptophan monohydrate was obtained from Calbiochem Co. and α-ethyl-3-hydroxy-4-methyl phenylethylamine (H75/12) from Aldrich Chemical Co. The test compounds were synthesized by Drs. A. A. Asselin, C. A. Demerson and I. Jirkovsky, Chemistry Department, Ayerst Laboratories. The doses refer to the salt of the compound.

RESULTS

Inhibition of [^{3}H]NE uptake. In Tables 1 and 2 are shown the ED_{50}'s of various test compounds and tricyclic antidepressant drugs on the uptake of [^{3}H]NE in the mouse heart. Compounds which did not cause a decrease in [^{3}H]NE levels of at least 50 per cent at 10 mg/kg, i.p., were not usually examined further in this assay. Those compounds which did exhibit this activity were further examined and their ED_{50}'s determined as were their effects on the release of [^{3}H]NE in the mouse heart. The effects of tandamine (compound 17) and the reference drugs desimipramine, imipramine and amitriptyline are shown on the uptake in Fig. 1A and release in Fig. 1B of [^{3}H]NE as representatives of results obtained in these assays. The relative activities of the reference agents were: desimipramine > nortriptyline = imipramine > amitriptyline (Table 1).

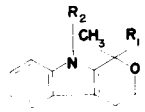
Compounds 1, 3, 8, 9, 10, 13 and 14, members of the pyranoindole series, like the reference tricyclic antidepressants, caused a decrease (ED_{50} = 10 mg/kg) when given before, but not after the [^{3}H]NE, thus indicating a blockade of the [^{3}H]NE uptake by these compounds (Table 1). In this series introduction of an ethyl group on the indole nitrogen in the compound containing the 2-dimethylaminoethyl side chain (1) yielded a compound (13) displaying the greatest blockade of the NE membrane pump, the level of activity being three times greater than imipramine. This appears to be the optimal length of the alkyl chain on the indole nitrogen since the compounds containing a propyl (14) or methyl group (9), or the unsubstituted compound (1), were greater than, or equivalent to imipramine, in potency, but less than compound 13.

The importance of the 2-dimethylaminoethyl side chain in compounds 1 and 9 is demonstrated by the finding that increasing the length by one carbon, i.e. 2 and 7, respectively, caused loss of activity; however, when in addition the nitrogen was secondary instead of tertiary, i.e. 3 and 8, respectively, the derivatives were similar in activity. Further, the activity was also lost when the replacement group was a 2-methylaminoethyl (4 and 12) or a 2-diethylaminoethyl alkyl chain (5 and 6). The placing of a 2-dimethylaminoethyl group on the indole nitrogen with the group at position 1 being a methyl (15) or propyl (16) yielded inactive compounds.

Compounds 10 and 11, enantiomers of compound 9, exhibited different activities, the (−)-enantiomer (10) being about three times more potent than the (+)-enantiomer (11) and the latter being similar in activity to the racemate, compound 9. These findings demonstrate the importance of the stereochemical configuration for biological activity.

Members of the thiopyrano indole series, i.e. 17, 18, 20, 21, 22, 23, 25, 26, inhibited the uptake of [^{3}H]NE as they caused a decrease in [^{3}H]NE when given before, but not after, the [^{3}H]NE (Fig. 1; Table 2). Enhanced activity is achieved by replacement of the oxygen (1, Table 1) by a sulfur atom (20, Table 2) in the compound containing an unsubstituted indole nitrogen and the 2-dimethylaminoethyl side chain (i.e. ED_{50}: 7.3 mg/kg, i.p. vs 2.4 mg/kg, i.p., respectively). The importance of the substituent on the indole nitrogen in the thiopyranoindoles is shown by

Table 1. Effects of pyrano [3,4-b]indoles and reference drugs on heart [^{3}H]NE uptake and the 5-HTP syndrome in mouse



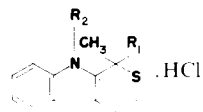
Treatment	R_1	R_2	Form	Inhibition of [^{3}H]NE uptake* ED_{50}: mg/kg, i.p.	Potentiation of 5-HTP syndrome† Dose (mg/kg, i.p.) 25	12.5	6.25
1	$CH_2CH_2N(CH_3)_2$	H	$.C_2H_2O_4$	7.3	I		
2	$CH_2CH_2CH_2N(CH_3)_2$	H	$.C_2H_2O_4$	>10	I		
3	$CH_2CH_2CH_2NHCH_3$	H	$.C_2H_2O_4$	8.2	I		
4	$CH_2CH_2NHCH_3$	H	$.C_4H_4O_4$	>10	I		
5	$CH_2CH_2N(CH_2CH_3)_2$	H	.HCl	>10	+1		
6	$CH_2CH_2N(CH_2CH_3)_2$	CH_3	.HCl	>10	I		
7	$CH_2CH_2CH_2N(CH_3)_2$	CH_3	$.C_4H_4O_4$	>10(11.5)	I		
8	$CH_2CH_2CH_2NHCH_3$	CH_3	$.C_4H_4O_4$	4.7	I		
9‡	$CH_2CH_2N(CH_3)_2$	CH_3	.HCl	3.7	+3	+2	+1
10	(−)-$CH_2CH_2N(CH_3)_2$	CH_3	.HCl	3.0	+3	+2	+1
11	(+)-$CH_2CH_2N(CH_3)_2$	CH_3	.HCl	10.5	+1		
12	$CH_2CH_2NHCH_3$	CH_3	.HCl	>10	I		
13	$CH_2CH_2N(CH_3)_2$	CH_2CH_3	.HCl	1.5	+1		
14	$CH_2CH_2N(CH_3)_2$	$CH_2CH_2CH_3$	$.C_4H_4O_4$	3.0	+1		
15	CH_3	$CH_2CH_2N(CH_3)_2$	$.C_4H_4O_4$	>10	I		
16	$CH_2CH_2CH_3$	$CH_2CH_2N(CH_3)_2$	$.C_2H_2O_4$	>10	I		
Imipramine				5.5	+3	+2	+1
Desimipramine				1.0	+1		
Amitriptyline				7.2	+4	+3	+2
Nortriptyline				4.4	I		

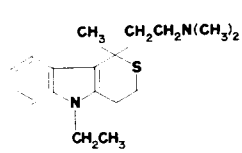
* Mice were administered [^{3}H]NE (2.5 μCi, i.v.) 45 min after the test agent. Animals were killed 2 hr after the latter treatment. The ED_{50} for inhibition of [^{3}H]NE uptake is derived from at least 3 doses for each agent as shown in Fig. 1 for tandamine and reference drugs.

† Mice were injected with compounds 30 min before an i.p. injection of 5-hydroxytryptophan (5-HTP; 300 mg/kg) and the syndrome scored as I (ineffective), +1 (weak effect), +2 (moderate effect), +3 (strong effect), +4 (very strong effect) 15 min after the 5-HTP injection.

‡ 1,9-Dimethyl-1-[2-(dimethylamino)ethyl]-1,3,4,9-tetrahydro-pyrano [3,4-b]indole hydrochloride.

Table 2. Effects of thiopyrano [3,4-b]indoles and structurally-related agents on heart [^{3}H]NE uptake and the 5-HTP syndrome in mouse



Treatment	No.	R_1	R_2	Inhibition of [^{3}H]NE uptake* ED_{50}: mg/kg, i.p.	Potentiation of 5-HTP syndrome† Dose (mg/kg, i.p.) 25	12.5	6.25
Tandamine‡	17	$CH_2CH_2N(CH_3)_2$	CH_2CH_3	0.3	+1		
(−)-enantiomer	18	$CH_2CH_2N(CH_3)_2$	CH_2CH_3	0.17	+2		
(+)-enantiomer	19	$CH_2CH_2N(CH_3)_2$	CH_2CH_3	>5	I		
	20	$CH_2CH_2N(CH_3)_2$	H	2.4	I		
	21	$CH_2CH_2N(CH_3)_2$	CH_3	1.0	+3	+2	+1
	22	$CH_2CH_2N(CH_3)_2$	$CH_2CH_2CH_3$	2.0	+1		
	23	$CH_2CH_2NHCH_3$	CH_2CH_3	2.3	+2		
	24	$CH_2CH_2NH_2$	CH_2CH_3	>10	+2		
	25	$CH_2CH_2N(CH_3)CH(CH_3)C_6H_4Cl$ (O)	CH_2CH_3	7.2	+1		
	26	$CH_2CH_2N(CH_3)_2$	$CH_2CH=CH_2$	2.5	+1		
	27			>10	+3	+1	+1

* † See legend to Table 1 for experimental details.

‡ 1-[2-(Dimethylamino)ethyl]-9-ethyl-1,3,4,9-tetrahydro-1-methyl-thiopyrano [3,4-b]indole hydrochloride.

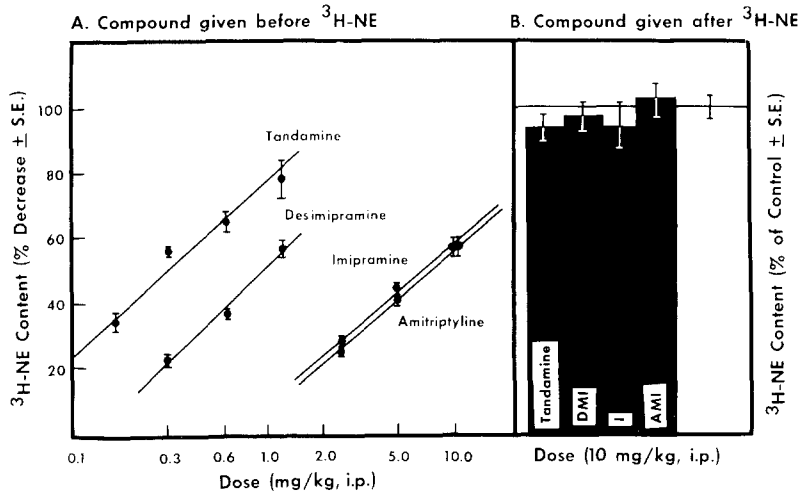


Fig. 1. Mice were administered [^{3}H]NE (2.5 μCi, i.v.) either 45 min before (Fig. 1A) or after (Fig. 1B) the test agent. Animals were killed 2 hr after the latter treatment. Each value is mean + S.E.M. of at least 8 animals.

the findings that the compound containing a methyl group (21) is more active (ED_{50}: 1.0 mg/kg, i.p.) than the unsubstituted derivative (20, ED_{50}: 2.4 mg/kg, i.p.). Furthermore, as in the pyrano indole series, the most effective compound in the series, i.e. tandamine (17), ED_{50}: 0.3 mg/kg, i.p., contains an ethyl group on the indole nitrogen; the activity was about eighteen times that of imipramine and five times that of the corresponding pyrano indole, compound 13 (Table 1). (Tandamine was also highly effective when administered orally, being one-half as active as intraperitoneally: ED_{50}: 0.6 vs 0.3 mg/kg, i.p.). Increasing the length of the substituent on the indole nitrogen to that of a propyl (22) or an allyl group (26) led to a decrease in activity; the unsaturation in the latter substituent was not of relevance as these two compounds were similar in activity. The significance of the dimethylamino group in the dimethylaminoethyl side chain at position 1 of this series was demonstrated, since replacement by a methylamino (23), a *p*-chloro-phenacyl-methylamino (25) or an amino group (24) led to a decrease in potency, in that order, as compared to tandamine (17).

Resolution of the racemate, tandamine, into its enantiomers was of importance as the (−)-enantiomer, 18, was the most potent compound of those examined; it was about twice as active as tandamine and at least thirty times as active as the (+)-enantiomer, 19, indicating the relevance of the sterochemical configuration for biological activity.

In the thiopyrano [4,3-b]indole series, instead of the thiopyrano [3, 4-b]indole series, compound 27, containing the dimethylaminoethyl side chain, was inactive.

Potentiation of the 5-HTP-induced behavioural syndrome. Sub-threshold behavioural doses of 5-HTP have been shown to be potentiated by certain tricyclic antidepressants [10, 11]. The degree of potentiation of the syndrome produced by 5-HTP for compounds examined and the reference drugs are presented in Tables 1 and 2. Only compound 9, a pyranoindole, and 21, a thiopyranoindole, displayed an activity with a potency at least similar to that of imipramine, the second most effective reference drug. Amitriptyline was the most effective in this respect whereas desimipramine was relatively inactive.

With respect to the pyranoindoles the importance of the 2-dimethylaminoethyl side chain was shown by the findings that, in contrast to the compound which contains this chain and also a methyl on the indole nitrogen (9), the compounds in which the dimethylamino group was substituted by a diethylamino (6) or the chain was lengthened to the 3-dimethylaminopropyl (7) or the 3-methylaminopropyl group (8) were ineffective. The significance of the nature of the substituent on the indole nitrogen was demonstrated by the findings that the activity was lost, or greatly reduced, when the methyl group of compound 9 was replaced by a hydrogen (1), ethyl (13) or propyl group (14) whether in the hydrogen-containing compound (1) the side chain was 2-aminomethyl (4) or 2-diethylaminoethyl (5), 3-dimethylamino (2) or 3-methylaminopropyl (3).

Similar to the findings obtained on the ability to block the uptake of [^{3}H]NE, the (−)-enantiomer (10) was highly effective, retaining the same degree of 5-HTP-potentiating activity as the racemate (9) whereas the (+)-enantiomer (11) was only weakly effective.

In the thiopyranoindoles, compound 21 (Table 2) produced potentiation of 5-HTP equivalent to that of imipramine. Thus, the oxygen in the pyrano indole (9, Table 1) can be replaced by a sulfur atom. However, the presence of the methyl group on the indole nitrogen is of relevance, since the compounds containing an ethyl (17), propyl (22), or allyl (26), group or a hydrogen atom (20) were relatively ineffective. This was also observed when the substituent on the indole nitrogen was an ethyl group, and the side chain, instead of the 2-dimethylaminoethyl (17), was a 2-methylamino-(23), amino-(24) or a *p*-chloro-phenacyl-methyl-amino (25)-ethyl group. The compound having the thiopyrano [4,3-b]indole (27), instead of the thiopyrano [3,4-b]indole (17), exhibited comparable activity at the highest dose, but not at the lower dose. Neither enantiomer of tandamine (17) potentiated 5-HTP strongly, but as observed with their effect on [^{3}H]NE uptake, the (−)-enantiomer (18) was rela-

Table 3. Effects on reserpine-induced hypothermia in mouse

Treatment	Dose (mg/kg, i.p.)	Agent alone	Temperature hr after reserpine 3	4	5
Tandamine	10	-	3+	4+	4+
	5	-	3+	4+	4+
	1	-	2+	2+	2+
Compound 9	20	-	2+	3+	3+
	10	-	2+	2+	2+
	2.5	-	1+	-	-
Desimipramine	10	-	2+	3+	4+
	5	-	2+	3+	3+
	1	-	-	2+	2+
Imipramine	20	(−)1+	2+	2+	3+
	10	-	1+	2+	2+
	2.5	-	-	-	-
Amitriptyline	20	(−)4+	2+	3+	3+
	10	(−)2+	2+	2+	2+
	2.5	-	1+	-	-

Mice (10 per group) received test agents 1 hr before administration of reserpine (1 mg/kg, s.c.). Temperatures were measured before test agents and reserpine and thereafter up to 5 hr.

Score $P < 0.05$: difference between mean experimental and mean control temperature: 1+, < 1 ; 2+, 1–2 ; 3+, 2–3 ; 4+, > 3 . Mean control temperatures of saline-reserpine group at 3, 4 and 5 hr after reserpine were 32.9 ± 0.36, 31.7 ± 0.30 and 31.5 ± 0.51 , respectively.

tively more active than the (+)-enantiomer (19) or the recemate (17).

Effect on reserpine-induced hypothermia. A compound of each series, i.e. compound 9 (Table 1) and tandamine (17, Table 2), was compared to desimipramine, imipramine and amitriptyline for its effect on reserpine-induced hypothermia. Each of the agents in doses ranging from 1 to 20 mg/kg, i.p., antagonized the reserpine-induced hypothermia at 3, 4 and 5 hr after the reserpine (Table 3). (Little or no change was seen in the temperature 1 or 2 hr following reserpine). The antagonism was dose-dependent. The order of activity was tandamine > desimipramine > compound 9 ≈ imipramine. Amitriptyline antagonized the hypothermia; however, alone it lowered the rectal temperature significantly in contrast to the other

Table 4. Effects on the guanethidine-induced displacement of [^{3}H]NE

Treatment	Dose (mg/kg, i.p.)	Agent alone (cpm × 10^3/g heart ± S.E.M.)	Agent + guanethidine	Per cent. inhibition*	ED_{50} (mg/kg, i.p.)
Saline		34.2 ± 0·90	12.6 ± 0.41		
Tandamine	5	50.1 ± 1.77	41.1 ± 1.57	72	2.1
	2.5	43.0 ± 2.71	30.8 ± 1.69	55	
	1.25	43.4 ± 2.45	25·5 ± 2.08	35	
	0.625	40.2 ± 0.91	18.8 ± 2.38	16	
Compound 9	20	41.7 ± 2.75	28.3 ± 1.74	49	≈ 20
	10	44.3 ± 2.65	25.0 ± 2.12	31	
	5	41.0 ± 2.98	20.4 ± 1.59	21	
Desimipramine	5	34.2 ± 3.40	29.6 ± 3.00	79	2.6
	2.5	34.3 ± 2.00	21.3 ± 3.30	40	
	1.25	35.7 ± 2.80	20.7 ± 0.98	34	
Imipramine	20	48.8 ± 5.14	31.6 ± 1.98	44	> 20
	10	41.2 ± 2.49	24.4 ± 1.74	36	
	5	41.0 ± 1.91	16.1 ± 1.12	4	
Amitriptyline	20	56.2 ± 4.55	43.0 ± 2.20	63	15
	10	38.6 ± 1.73	22.5 ± 1.84	34	
	5	34.6 ± 2.56	18.4 ± 1.12	26	
Butriptyline	20	42.4 ± 3.40	18.5 ± 0.70	11	> 20
	10	34.3 ± 2.88	16.2 ± 1.65	16	
	5	32.4 ± 2.18	15.8 ± 1.09	19	

The animals were killed 4 hr after the i.v. administration of [^{3}H]NE. Guanethidine (20 mg/kg, i.p.) was given 45 min after the test agents and saline injections, which were given 75 min following [^{3}H]NE. Each value is mean ± S.E.M. of at least 6 mice per group. ED_{50} is dose at which agents inhibited the depletion of [^{3}H]NE by 50 per cent. * Per cent inhibition was calculated by formula stated in methods.

Table 5. Effects on H75/12-induced displacement of 5-hydroxytryptamine (5-HT) in mouse brain

Treatment	First dose (mg/kg, i.p.)	Brain 5-HT (μg/g ± S.E.M.)		Per cent inhibition§
		Drug alone	Drug + H75/12	
Saline		0.75 ± 0.02	0.40 ± 0.02*	
Compound 9	25	0.74 ± 0.01	0.70 ± 0.03‡	87
Saline		0.67 ± 0.01	0.40 ± 0.02	
Compound 21	25	0.62 ± 0.01	0.50 ± 0.02†	52
Saline		0.72 ± 0.05	0.48 ± 0.02	
Imipramine	25	0.73 ± 0.06	0.61 ± 0.02‡	51

Mice were injected with two doses of α-ethyl-3-hydroxy-4-methyl phenylethylamine (H75/12; 100 mg/kg, i.p.) 2 hr apart. All animals were killed 2 hr after the last dose of H75/12. Test agents were injected 30 min prior to H75/12, the second dose being half the first. Each result is mean of 5–7 animals.

* $P < 0.001$ with respect to saline.

† $P < 0.01$; ‡ $P < 0.05$ with respect to saline + H75/12.

§ Per cent inhibition was calculated by formula stated in methods.

agents. Thus, a comparison of the effects of amitriptyline with the other agents is difficult.

Effect on guanethidine-induced displacement of [^{3}H]NE. The agents examined with respect to their effects on the reserpine-induced hypothermia were studied as to their effects on the guanethidine-induced displacement of previously administered [^{3}H]NE. Butriptyline, another tricyclic antidepressant [15], was also examined. The guanethidine-induced displacement of the [^{3}H]NE was prevented by various of the agents in a dose-related manner with the order of activity and ED_{50}'s (mg/kg, i.p.) being tandamine (2.1) > desimipramine (2.6) > amitriptyline (15) > compound 9 (≈ 20) > imipramine (> 20) (Table 4). Butriptyline was ineffective when studied at doses at which the other agents exhibited activity.

Effect on displacement of 5-HT by H75/12. H75/12 caused a depletion of mouse brain 5-HT levels (Table 5). Compounds 9, 21 and imipramine (25 + 12.5 mg/kg, i.p.) antagonized the H75/12-induced depletion, with compound 21 exhibiting a level of activity equivalent to imipramine; the effect of compound 9 was greater than either imipramine or compound 21. No alterations in 5-HT levels occurred when the agents were administered alone.

DISCUSSION

The present findings indicate that certain pyrano [3,4-b]indoles and thiopyrano [3,4-b]indoles are blockers of NE and/or 5-HT uptake *in vivo*, and in addition, those which block [^{3}H]NE uptake exhibit the ability to antagonize reserpine-induced hypothermia and guanethidine-induced displacement of [^{3}H]NE from heart. Such activities are in accord with the hypothesis that drugs which block the inactivation of catecholamines and/or 5-HT through blockade of their uptake at the respective NE and/or 5-HT neurons can cause potentiation of their effects [6, 16].

Activity with respect to the inhibition of the [^{3}H]NE uptake is shown by compounds containing the pyranoindole ring system which is substituted with two substituent groups in position 1, i.e. with a methyl group and with a 2-dimethylaminoethyl or 3-methylaminopropyl groups. Substituting the ring oxygen with a sulfur atom in the 2-dimethylaminoethyl compound results in enhanced activity (i.e. tandamine). This latter observation is of interest as such a change in a bicyclic series leads, in contrast, to a decrease in activity, i.e. Lu 3-010 vs Lu 5-003 [17]. In the pyrano-indole series containing the 2-dimethylaminoethyl side chain, alkylation of the indole nitrogen with an ethyl group yields the compound exhibiting the highest activity.

Similar structural elements were observed to be essential for [^{3}H]NE uptake in the thiopyrano-indole series. The presence of the thiopyrano group is much more favourable as the highest activity of all of the compounds examined in this study was exhibited by tandamine and its (−)-enantiomer-compounds containing this group. In this respect these latter compounds appear to be among the most potent agents known to block the NE membrane pump. The importance of the indole ring in the most active compounds in the thiopyrano and pyrano-indole series is indicated by the findings that replacement by an indene ring, i.e. in pirandamine, resulted in loss of the activity (W. Lippmann and T. Pugsley, in preparation).

The secondary methylamines were more potent blockers of NE uptake than the tertiary derivatives (i.e. dimethylamines) in the present studies, as observed with the 3-aminopropyl derivatives of the 9-methylpyrano indoles (8 vs 7, Table 1). This relative activity has also been demonstrated in other series such as the dihydrodibenzazepines, i.e. desimipramine vs imipramine [16, 18] and the 3,3-dimethyl-1-phenylphthalan-1-[3-aminopropyl] derivatives (Lu 3-010 vs Lu 3-009) [17]. In the present studies this relative activity was also observed in the dibenzocycloheptadiene (nortriptyline vs amitriptyline) and the dihydrodibenzazepines (desimipramine vs imipramine). In studies *in vitro* with the rabbit aortic strip [19, 20] and rat cerebral cortex slices [21] such relationships were observed with the secondary vs tertiary methylamines of the dihydrodibenzazepine, dibenzocycloheptadiene and diphenylmethylidene series. These findings thus demonstrate the similarity of the mouse heart *in vivo* system to the above two *in vitro* preparations with these drugs.

In contrast to the relative activities of the compounds mentioned above, the 2-aminoethyl derivatives of the pyrano-indoles exhibited a reverse structure activity relationship, i.e. the tertiary (1, Table 1) was more active than the secondary (4, Table 1) methylamine. Thus, the nature of the side chain, i.e. the 2-aminoethyl group, appears to be critical since

when, instead of the 2-aminoethyl group the chain is the aminopropyl, in the 9-methyl (8 vs 7, Table 1) and in the unsubstituted (2 vs 3, Table 1) derivatives, the secondary derivative is the more active. In accord are the findings that, of the 9-methyl pyrano-indoles and thiopyrano-indoles containing the 2-aminoethyl side chain, the tertiary is more active than the secondary (9 vs 12, Table 1; 17 vs 23, Table 2).

Maxwell *et al.* [19] suggested, in regard to space-filling molecular models of various series of tricyclic compounds, with respect to the blockade of the NE uptake mechanisms, that compounds in which the bridges between the two phenyl rings held the phenyls at angles to one another were potent, whereas those in which the rings were planar, or near planar, were considerably less potent. In the present studies the most potent blockers were either a thiopyrano-indole or a pyrano-indole; the compounds were more potent than imipramine. In these series the indole and the thiopyran or pyran ring are in a near planar configuration; therefore, this is in contrast to the angular configuration of the phenyls in imipramine. Thus, this indicates that other factors are of importance. The conclusions of Maxwell *et al.* [19] were based upon findings *in vitro* whereas the present studies were carried out *in vivo* and the availability of the active compounds would thus be a factor. With respect to the amine uptake mechanism in the rabbit aortic strip and that in the heart *in vitro*, based on the kinetics of NE uptake and the inhibitory effects of tricyclics and related compounds, Maxwell *et al.* [19] concluded that the amine uptake mechanisms were similar; Sakama *et al.* [21] reported that the amine uptake mechanism in the rabbit aorta and rat cerebral cortex *in vitro* preparations appeared to be fundamentally similar.

Pharmacologically, it has been shown that most clinically useful tricyclic antidepressants antagonize reserpine-induced hypothermia [22, 23]. This activity is generally considered to represent a central effect [24]. Tandamine and compound 9, like the known antidepressants examined when given before reserpine, antagonized the hypothermia produced by reserpine in a dose-related manner. Tandamine displayed the greatest activity in preventing the reserpine-induced hypothermia. The order of activity was similar to that found in blocking the [^{3}H]NE uptake. Such a relationship has also been observed with these agents when the *in vivo* rat brain NE uptake system was utilized (T. Pugsley and W. Lippmann, in preparation). Thus, this effect can be considered to be due to the ability of these agents to inhibit the re-uptake of NE that is released in the depleting process by reserpine, thus leading to higher levels of NE in the vicinity of the adrenergic receptors [24].

A number of agents, including some members of the tricyclic antidepressant group which block the NE membrane pump, have been shown, in addition, to inhibit the depletion of NE stores caused by guanethidine [25–27]. Further, these agents have been shown to antagonize the guanethidine-induced reduction of various responses to sympathetic nerve stimulation [26–28]. Guanethidine, when given after an i.v. injection of [^{3}H]NE, has been shown to increase the rate of the spontaneous release of labelled amine [29]. Tandamine and compound 9, similar to imipramine, desimipramine and amitriptyline, antagonized the guanethidine-induced decline in [^{3}H]NE levels. This antagonism is consistent with the hypothesis [30] that agents which block NE uptake also block the uptake of guanethidine into adrenergic neurons, as guanethidine utilizes the same membrane pump as NE. The lack of effect of butriptyline is consistent with this hypothesis as it did not exhibit an effect on the NE membrane pump [31]. In this respect, tandamine exhibited the strongest activity in antagonizing the guanethidine-induced displacement, in agreement with its activity in blocking the uptake of [^{3}H]NE.

A blockade of the 5-HT membrane pump is also considered to be of importance in the mechanism of action of certain tricyclic antidepressants [7, 32]. In the present study potentiation of the behavioural syndrome produced by 5-HTP was used to indicate the effects of various compounds upon 5-HT related activities [10, 11]. An effect in this test has been shown generally to correlate with the ability of a compound to inhibit the 5-HT membrane pump [11], provided such an agent is neither a monoamine oxidase (MAO) inhibitor nor a releaser of serotonin [7]. Further, a potentiation of a subthreshold dose of 5-HTP appears to reflect mainly an action on brain 5-HT neurons [33, 34]. In the present study, imipramine, but not desimipramine, potentiated strongly the 5-HTP-induced syndrome; these findings are thus consistent with those of Carlsson *et al.* [10, 35]. It is of interest that amitriptyline exhibited even more potentiation than imipramine.

From the present studies, optimal potentiation of 5-HTP in both the pyrano and thiopyrano series is achieved when there is present at position 1 a methyl group and a 2-dimethylaminoethyl alkyl side chain with a methyl group on the indole nitrogen. It is of interest that enhanced activity is obtained by replacement of the indole ring by an indene ring, with the same groups at position 1 of the pyrano ring, yielding a compound, pirandamine, which like amitriptyline maximally potentiates 5-HTP and is relatively ineffective in blocking NE uptake (W. Lippmann and T. Pugsley, in preparation).

Both compounds, i.e. 9 and 21 which, like imipramine, exhibited optimal 5-HTP potentiating activity, also antagonized the H75/12-induced depletion of brain 5-HT. Such an antagonism is considered to reflect a blockade of the 5-HT membrane pump as H75/12 must be taken up by this pump in order to cause depletion of brain 5-HT [7]. Thus, the biochemical findings correlate with the functional findings. As these compounds do not inhibit monoamine oxidase (T. Pugsley and W. Lippmann, in preparation), the present findings support the conclusion that they are blocking the 5-HT membrane pump.

The structural requirements for blockade of the 5-HT membrane pump shows some general characteristics similar to those for the NE pump but various striking differences exist. Imipramine and amitriptyline blocked both, with these drugs generally considered to exhibit a higher activity on 5-HT uptake [7]; desimipramine did not exhibit appreciable activity on the 5-HT pump, but was potent in its action on the NE pump. Thus, in contrast to NE uptake, the presence of a tertiary nitrogen is more favourable than the secondary for a blockade of 5-HT uptake. A similar relationship between the compound 9 and

the secondary derivative compound 12 is apparent in the present study.

As with the blockade of [^{3}H]NE uptake, the nature of the substituent on the indole nitrogen was of relevance for high 5-HT uptake blockade activity in the pyrano and thiopyrano-indoles. However, the two systems differ as, in contrast to the ethyl group for maximal NE uptake blockade, the methyl group yielded the most active compound for 5-HT uptake blockade. In these two series, each of the most active compounds on the 5-HT pump, like imipramine, exhibited blocking action on both the 5-HT and NE membrane pump, whereas the most active NE pump blockers tandamine (17) and compound 13 displayed only weak effects on the 5-HT pump.

The resolution of the enantiomers of biologically active compounds has proved to be of importance [36, 37]. The present studies further substantiate this finding. The (−)-enantiomer of tandamine and compound 9 proved either to be greater with regard to inhibition of the NE uptake or equivalent for the inhibition of 5-HT uptake to the racemate, whereas the (+)-enantiomer was ineffective or less effective.

NE- and 5-HT-containing neurons have been implicated in a variety of centrally mediated effects, for example sleep [38], thermoregulation [39], behaviour [40, 41] and depression [41–43]. With respect to depression, an increase in drive or psychomotor activation in depressed patients has been correlated with an inhibition of brain NE uptake, e.g. desimipramine and protriptyline, whereas a brightening of mood in such patients has been related to an inhibition of 5-HT uptake, e.g. imipramine and amitriptyline [7, 8]. In each case, the net effect is probably an increase in the levels of NE or 5-HT at critical receptor sites in the central nervous system and this is considered to be one of the important factors in the antidepressant action of such drugs in humans [44]. Agents such as tandamine, which is shown in the present study to be one of the most potent and selective NE membrane pump inhibitors known, exhibiting no appreciable effect on the 5-HT membrane pump, should be useful for studying various physiological activities of NE. Where an action on both NE and 5-HT membrane pumps is desired, compounds 9 or 21, which respectively exhibited activity either greater than or equivalent to imipramine, would be useful. In this respect, tandamine is currently of interest clinically for use as an antidepressant drug.

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