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December 28, 1966

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Adamantanamines and their derivatives as sensitizing agents for 5-hydroxytryptamine-induced contraction of smooth muscle

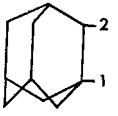
SIR,—It has been shown *in vitro* that smooth muscle contractions induced by 5-hydroxytryptamine (5-HT) or noradrenaline can be sensitized with different drugs. Sigg, Soffer & Gyermek (1963) reported the sensitizing effect of imipramine on the 5-HT- and noradrenaline-induced contraction of the nictitating membrane of the cat. Rossum (unpublished) found that the log concentration-response curve of noradrenaline is shifted to lower concentrations if imipramine or cocaine is used as sensitizer. Offermeier (1965) has described an increase in the response of the rat fundus strip to 5-HT and of the rat vas deferens to noradrenaline after preincubation with imipramine or cocaine. Using cocaine, with some preparations shifts have been obtained with a factor of almost 100. Continuing our study of receptors of neurotransmitters (Wesemann & Zilliken, 1966) we now describe the influence of adamantanamines and their derivatives on 5-HT-induced contractions of the rat isolated fundus strip.

Male rats, strain Wistar II, 160–180 g, starved for 48 hr but given water *ad libitum*, were used for the fundus strip preparation according to Vane (1957). The mucosa was carefully removed to facilitate the washing out of drugs. The muscle strip was incubated in oxygenated Tyrode (10 ml) at 37°. The strip was fixed to one end of a lightly loaded isotonic lever giving about 20 times magnification. Cumulative dose-response curves were obtained by gradually increasing the dose without washing out (Ariëns & de Groot, 1954).

The antiviral compound amantadine (adamantan-1-amine) inhibits the penetration of influenza A_2 virus into the cell (Davies, Grunert, Haff & others, 1964). We were unable to demonstrate a significant inhibition of influenza virus neuraminidase (A_2-Japan virus 1957 E.C.3.2.1.18). However amantadine, in concentrations higher than 10^{-5} M, sensitizes the rat fundus strip to 5-HT (tested with J. Offermeier). With a concentration of 10^{-4} M of the compound the dose-response curve for 5-HT is shifted to the left by a factor of about 10. Maximal sensitization (usually a factor of about 100) is achieved with 10^{-3} M

amantadine. With higher concentrations the ability of the fundus strip to contract in response to 5-HT is lost and cannot be restored by washing out the drugs.

TABLE 1. EFFECT OF ADAMANTANE DERIVATIVES ON THE LOG DOSE-RESPONSE CURVE OF 5-HT OBTAINED ON THE RAT FUNDUS STRIP



Compound	Side-chain	Molarity	Sensitization	α*	No. Strips	No. Curves
Adamantan-1-amine HCl	1-NH_2·HCl	10^{-4} 10^{-3}	10 100	—	58	170
Adamantan-2-amine HCl	2-NH_2·HCl	10^{-4}	150	—	12	62
N-Ethyladamantan-1-amine HBr	1-NH·Et·HBr	10^{-4} 3×10^{-4}	10–15 100	—	15	64
N-(Adamant-1-yl)urea	1-NH·CO·NH_2	10^{-8} 10^{-7} 10^{-6}	10 60–80 500	—	18	54
N-(Adamant-1-yl)-isocyanate	1 — N:C:O	10^{-6}	—	—	13	42
N-(Adamant-1-yl)-isothiocyanate ..	1 — N:C:S	10^{-7}	10	—	9	31
4-(*N*-Adamant-1-yl-carbonyl)morpholine**	1-NH·CO-N⟨O⟩	10^{-8} 10^{-5}	— —	1·25 —	12	54
N-(Adamant-1-yl)-*N'*-cyclohexylurea** ..	1-NH·CO·NH·⟨⟩	10^{-9} 10^{-6} 10^{-5}	— — —	1·15 0·6 0·4	16	64
N-(Adamant-1-yl)-N'-phenylurea** ..	1-NH·CO·NH·Ph	10^{-8} 10^{-6} 10^{-5}	— — —	1·1 0·7 0·3	12	51
1,1-Diethylpropylamine		10^{-4}	—	—	9	36

* Relative intrinsic activity. Adamantane compounds were added in Tyrode solution, or where necessary in propylene glycol/Tyrode of ethylene glycol monoethyl ether. Final concentration maximal 0·1% v/v.
** Schlatmann, J. L. M. A. & Schuti, J., to be published.

Table 1 summarizes the sensitizing effect of 12 adamantane compounds tested on the rat fundus strip. 1,1-Diethylpropylamine has been included because of some structural relationship with amantadine. These substances have been synthesized as potential antiviral compounds. Fig. 1 shows a log dose-response curve for 5-HT tested on the rat fundus preparation in the presence of different concentrations of *N*-ethyladamantan-1-amine hydrobromide. Since it is not known how the active adamantane compounds cause sensitization, we prefer to express the shift of the 5-HT log concentration-response curve as a factor rather than as a change in affinity $1/K_A$, the value K_A being the dissociation

constant of the drug-receptor complex. The log dose-response curve obtained resembles the type for a competitive synergism more than the theoretical dose-response curve for an agonist A combined with various concentrations of a sensitizing compound B (Ariëns, Simonis, & Rossum, 1964). In the latter case, a parallel shift to the left would be expected when increasing concentrations of B (adamantane compounds) are used, until a limit is reached.

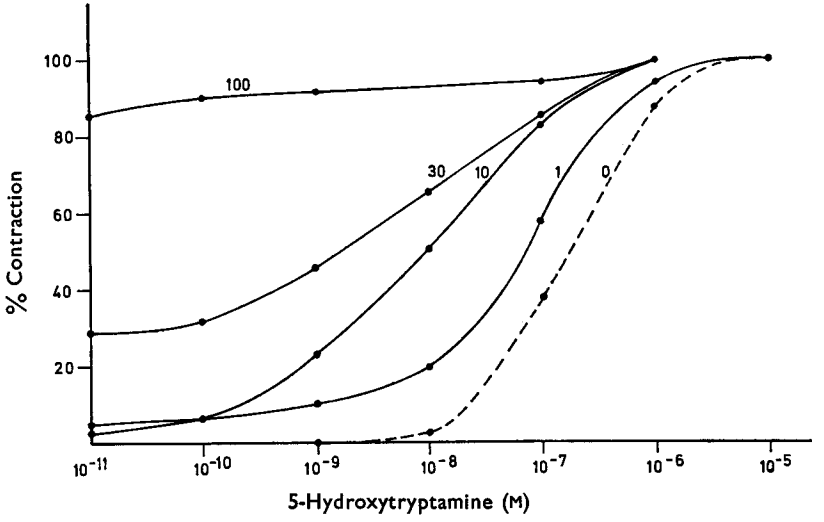


FIG. 1. Cumulative log dose-response curves of 5-HT in the presence of various concentrations ($\times 10^{-5}$M) of *N*-ethyladamantan-1-amine HBr tested on the rat fundus preparation.

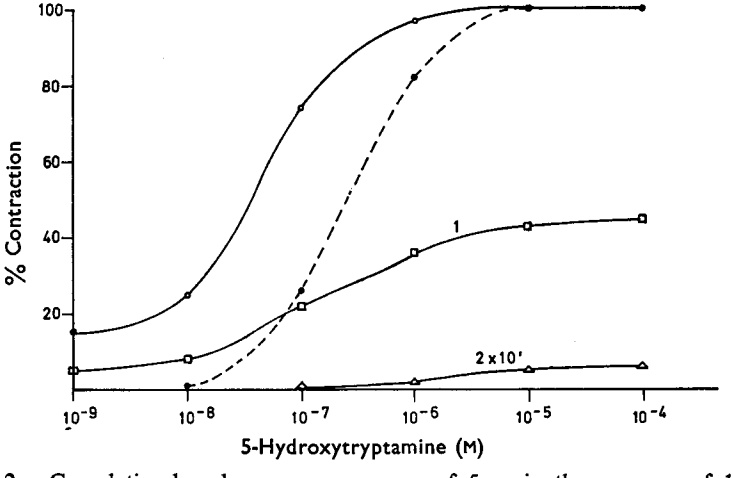


FIG. 2. Cumulative log dose-response curves of 5-HT in the presence of 10^{-4}M adamantan-1-amine after various incubation times with 3×10^{-4} dibenamine, tested on the rat fundus strip preparation. 5-HT log dose-response curve ●-----●; 5-HT log dose-response curve sensitized by 10^{-4}M adamantan-1-amine after incubation with 3×10^{-4} dibenamine for 0 min ○——○, 5-HT log dose-response curve sensitized by adamantan-1-amine after incubation with 3×10^{-4}M dibenamine; incubation time 10 min □——□, incubation time 20 min △——△.

Some of the compounds tested, though inactive as sensitizers, influence the relative intrinsic activity α. With *N*-(adamant-1-yl)-*N'*-cyclohexylurea the maximum height of the 5-HT dose-response curve is increased at low concentrations of the adamantane compound ($\alpha = 1{\cdot}15$). A decrease of the maximum height to 40% of the height of the curve for 5-HT alone occurs at a concentration of 10^{-5} M. Cocaine and imipramine produce sensitization to 5-HT in concentrations between 10^{-9} and 3×10^{-7} M and 10^{-8} and 10^{-6} M respectively (Offermeier, 1965). Log concentration-response curves for 5-HT on preparations already sensitized by adamantane compounds can be shifted further towards lower concentrations by incubating the fundus strip with 10^{-8} and 3×10^{-8} M cocaine (Table 2) whilst with imipramine no significant further increase or decrease could be obtained.

TABLE 2. EFFECT OF COCAINE ON THE LOG DOSE-RESPONSE CURVE OF 5-HT ON THE RAT FUNDUS STRIP* SENSITIZED BY ADAMANTANE COMPOUNDS

Compound	Concentration M	Concentration cocaine M	Sensitization factor†
Adamantan-1-amine HCl	10^{-5}	3×10^{-8}	10
N-Ethyladamantan-1-amine HBr	10^{-4}	10^{-8}	10
N-(Adamant-1-yl)urea	10^{-7}	10^{-8}	10

* Each experiment used 6 strips.

† Additional sensitization by cocaine of the 5-HT response already sensitized the adamantane compounds. Substances were added in Tyrode solution, only *N*-(adamant-1-yl)urea was dissolved in ethylene glycol monoethyl ether, final concentration maximal 0.1% v/v.

According to Woolley (1958) the 5-HT-receptor interaction opens a "valve" for the Ca-ion transport, which leads to the contraction of muscle. Contractions produced by just adding Ca^{2+} or Mg^{2+} ions in the rather high concentrations from 10^{-3} to 10^{-1} M to the organ bath were not influenced by dibenamine hydrochloride which irreversibly blocks 5-HT receptors. Since Ca-induced contractions are not influenced by dibenamine, this blocking agent may act directly on the 5-HT receptor and not interfere with the stimulus-event chain, that is to say, the Ca-ion transport, the polarization, depolarization, and the effector system. Inasmuch as the 5-HT concentration-response curves of the tissue sensitized by adamantanamine were found to decline after various incubation times with dibenamine (Fig. 2), adamantanamine apparently acts at the receptor level and not at the stimulus-effect chain. Perhaps the adamantane compounds react with storage sites of 5-HT or influence the drug-receptor metabolism thus increasing the concentration of 5-HT in the vicinity of the receptor.

Acknowledgements. We thank Miss H. Burkhardt and Miss I. Gipper for skilful technical assistance, Dr. Schlatmann, N. V. Philips-Duphar, for adamantanamine derivatives, and Dr. J. Offermeier for valuable discussions.

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December 6, 1966

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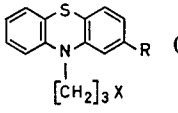
Activity correlations and the mode of action of aminoalkylphenothiazine tranquillizers

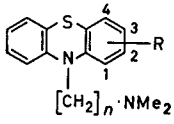
SIR,—Potent tranquillizing activity in the aminoalkylphenothiazine series is generally limited to those compounds (I) which have a substituent in the 2-position of the phenothiazine nucleus, and in which the phenothiazine nitrogen



(I) R = H, Cl, CF_3 etc.
X = Tertiary amino-group

atom and the terminal amino-group are separated by a trimethylene chain (Gordon, Craig & Zirkle, 1964). The amino-group and part of the side-chain may be incorporated into a ring system such as piperidine. Within this restricted range of structural variation, numerous correlations have been reported between

TABLE 1. CATALEPTIC ACTION, SURFACE ACTIVITY, IONIZATION CONSTANT, WATER SOLUBILITY AND EFFECT ON ATPase OF DIMETHYLAMINOALKYLPHENOTHIAZINES



R	*n*	ED50 (mg/kg) for catalepsy	Surface-active concn (μM)	I50 (μM) for ATPase inhibition	pK_a	Solubility (μM) of free base in water
H	3	25	800	250	9·4	50
1—Cl	3	none at 50	500	150	9·4	12
2—Cl	3	4	240	80	9·3	8
3—Cl	3	none at 20	180	120	9·2	10
4—Cl	3	none at 50	300	50	9·2	11
2—CF_3	3	2·5	70	100	9·2	5
4—CF_3	3	none at 50	50	80	9·3	7
2—Cl	2	none at 20	200	150	8·6	15
2—Cl	4	none at 25	140	200	9·7	5

ED50 is the dose of drug required to cause catalepsy in 3 out of 6 mice 1 hr after intravenous injection (Taeschler & Cerletti, 1958).
The surface-active concentration is that required to lower the surface tension of 10 mM sodium phosphate (pH 6·97) by 5 dynes/cm when measured with a Du Noüy tensiometer and platinum ring.
A rat brain microsomal suspension treated with sodium deoxycholate was used as a source of $(Na^+ + K^+)$-activated ATPase (Järnfelt, 1964). I50 is the concentration of compound required to inhibit the ouabain-sensitive fraction of the activity by 50% when measured at 37° in 30 mM tris buffer (pH 7·5) in the presence of 20 mM KCl, 100 mM NaCl, 5 mM $MgCl_2$ and 2·5 mM ATP.
The pK_a was derived from the pH dependence of the water solubility (Green, 1967).