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The Action of 1,2,3,5,6,11*b*-Hexahydro-[1]benzothieno [3,2-g]indolizine Hydrochloride (ADT 16) on Peripheral and Central Responses Mediated by 5-Hydroxytryptaminergic and Adrenergic Systems of the Rat

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Abstract—Some acute pharmacological effects have been examined of racemic ADT 16 (1,2,3,5,6,11*b*-hexahydro[1]benzothieno[3,2-g]indolizine hydrochloride), on peripheral and central responses mediated by 5-HT and adrenergic systems in the rat. In-vitro, ADT 16 (10–1000 nM), similarly to mianserin, antagonized the inhibitory responses to B-HT 920 of the electrically-stimulated rat isolated prostatic vas deferens. High concentrations of ADT 16 (10 μM), also resembled those of mianserin by potentiating twitch responses to electrical stimulation of the tissue. Contractile responses to phenylephrine of rat isolated epididymal vas deferens were antagonized by ADT 16 (0·3–1 μM). In the rat stomach fundus strip, ADT 16 (1–3 μM) antagonized contractions due to 5-HT. ADT 16 (0·1–1 μM) had no effect on responses to acetylcholine of the guinea-pig isolated ileum. In-vivo, in spinalized, decerebrated rats, fenfluramine- or clonidine-induced facilitation of flexor reflex activity of the anterior tibialis muscle was attenuated by ADT 16 (3 and 10 mg kg^{-1}, i.v., and 3 mg kg^{-1}, i.v. respectively). In the anaesthetized rat, L-3,4-dihydroxyphenylalanine (L-dopa)- or L-5-hydroxytryptophan (L-5-HTP)-induced increases in the frequency of spontaneous twitches of the anterior digastricus muscle were attenuated by ADT 16 (1 and 3 mg kg^{-1}, i.v.; n = 4). It is concluded that ADT 16, similarly to mianserin, is a novel peripherally and centrally active antagonist of 5-HT and adrenergic responses in the rat.

ADT 16 (University of Tasmania: 1,2,3,5,6,11*b*-hexahydro-[1]benzothieno[3,2-g]indolizine hydrochloride) (Fig. 1) was synthesized as a derivative of the novel heterocyclic [1]benzothieno[3,2-g]indolizine system (Browne 1985). There is considerable interest in the pharmacological properties of such heterocyclic compounds as isosteres of biologically active indoles (Campaigne et al 1970; Bosin & Campaigne 1977).

Preliminary radioligand receptor-binding studies in these laboratories suggested that ADT 16 had marked affinity for 5-HT_2 receptors and α_1- and α_2-adrenoceptors.

In the present investigation, some peripheral and central pharmacological actions of ADT 16 on 5-HT and adrenergic neuronal systems in the rat were studied. Experiments were carried out to determine whether its systemic administration affected certain central nervous system (CNS)-mediated responses. The acute pharmacological profile of ADT 16 was compared with that of the antidepressant mianserin (Fig. 1), which is an antagonist at 5-hydroxytryptamine (5-HT) receptors and α_2-adrenoceptors in the rat (Van der Burg et al 1970; Vargaftig et al 1971; Brown et al 1980; Van Riezen et al 1981).

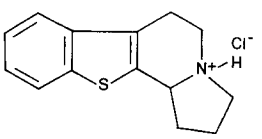
Materials and Methods

Chemistry

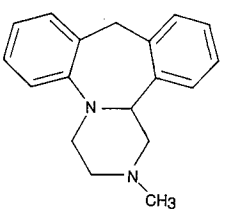
Racemic ADT 16 was synthesized as part of a synthetic programme (Department of Chemistry, University of Tasmania) aimed at finding a potent 5-HT-ergic/adrenergic antagonist by modification of the structure of 5-HT. The chemical synthesis of the free base of ADT 16 has previously been described and is the first reported example of the [1]benzothieno[3,2-g]indolizine system (Browne 1985), and the hydrochloride salt was made from the base by standard methods. In the present investigation, the corresponding hydrochloride salt of ADT 16 (synthesized by E. J. Browne) has been used.

In-vitro studies

Effects of ADT 16 and mianserin on the inhibitory effects of B-HT 920 on contractile responses of the rat isolated transmurally-stimulated prostatic vas deferens. Male Wistar rats, 150–



ADT 16



Mianserin

FIG. 1. The structure of ADT 16 and mianserin.

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300 g, were killed by cervical dislocation. Both vasa deferentia were isolated, bisected and the prostatic sections mounted individually in 15-mL organ baths containing Krebs–Henseleit solution (36°C, bubbled with 95% O_2–5% CO_2) of the following composition (mM): NaCl 118, KCl 4·70, $CaCl_2$ 2·54, KH_2PO_4 1·18, $MgSO_4$ 1·17, $NaHCO_3$ 24·88, glucose 5·55. Each vas deferens was equilibrated at a resting tension of 0·5 g for 30–45 min. Electrical field stimulation of the tissue was carried out by means of two platinum electrodes, one placed parallel alongside and the other inserted from the bottom vertically inside the lumen. Stimulus parameters were 0·2 Hz with square pulses of 1 ms duration at a supramaximal voltage (70 V). Grass force–displacement isometric transducers (FT03) and a Grass polygraph (79E) were used to record contractions.

Inhibitory concentration–response curves were generated by adding B-HT 920 cumulatively at 1-min intervals to the bath containing the electrically stimulated vas deferens. After an initial concentration–response curve to B-HT 920 had been obtained (control), the vas deferens was incubated with either ADT 16 or mianserin for 15 min and responses to cumulative concentrations of B-HT 920 again determined. Effects of higher concentrations of either ADT 16 or mianserin were similarly studied using the same tissue, after washing and restoration of the basal twitch response. Only one drug (ADT 16 or mianserin) was studied using any one tissue. Responses of the tissues to B-HT 920 were expressed as a percentage of the maximum inhibitory response to B-HT 920, obtained in the control. Concentration–ratio values were calculated from the IC50 (concentration of B-HT 920 causing a 50% inhibition of the twitch response in the absence or presence of ADT 16 or mianserin) and the data was used to generate a Schild plot (Arunlakshana & Schild 1959).

Effects of ADT 16 on contractile responses of rat isolated epididymal vas deferens to phenylephrine. The epididymal ends of the vasa deferentia described above were mounted individually in 15-mL organ baths containing Krebs–Henseleit solution (composition as above, 36°C, bubbled with 95% O_2–5% CO_2). The tissues were equilibrated under 0·5–0·75 g resting tension for 30–45 min before starting experiments. Contractions to phenylephrine were recorded using Grass force-displacement isometric transducers (FT03) and a Grass polygraph (79E). Concentration-response curves were obtained by adding phenylephrine cumulatively (Van Rossum 1963) to the organ bath. After an initial concentration-response curve was obtained (control), the tissue was then incubated for 15 min with ADT 16 and a second concentration–response curve to phenylephrine obtained. Subsequently, using further epididymal vasa deferentia, concentration–response curves to phenylephrine in the presence or absence of higher concentrations of ADT 16 were obtained similarly. Mean contractile responses were expressed as a percentage of the mean maximum control responses to phenylephrine.

Effects of ADT 16 on contractile responses to 5-HT of rat isolated stomach fundus strips. The method used was that described by Vane (1957). Male Wistar rats, 200–400 g, were killed by cervical dislocation and exsanguination and the pyloric antrum of the stomach removed. Strips (2 cm × 2 mm) were obtained by opening the fundus along its lesser curvature, preserving the longitudinal musculature. These were mounted in 15-mL organ baths containing Krebs–Henseleit solution (composition as above, 37°C, bubbled with 95% O_2–5% CO_2). Contractions to 5-HT were recorded isometrically by means of Grass force–displacement transducers (FT03) and a Grass polygraph (79E). Strips were equilibrated for 45–60 min before drugs were administered.

Concentration–contractile response curves were obtained by adding 5-HT cumulatively (Van Rossum 1963) to the organ bath. An initial control concentration–response curve was obtained. The strips were then incubated for 15 min with ADT 16, and a second concentration–response curve to 5-HT obtained. Subsequently, using separate stomach strips, concentration–response curves to 5-HT in the presence or absence of higher concentrations of ADT 16 were obtained similarly. Mean contractile responses were expressed as a percentage of the mean control maximum response to 5-HT.

Effects of ADT 16 on contractile responses of the guinea-pig ileum to acetylcholine. Male guinea-pigs were killed instantly by cervical dislocation and exsanguination. An approximate 2-cm length of ileum was removed and mounted in a 15-mL organ bath containing Tyrode solution (37°C, bubbled with 95% O_2–5% CO_2) of the following composition (mM); NaCl 136·89, KCl 2·68, $CaCl_2$ 1·80, NaH_2PO_4 0·4, $MgCl_2$ 1·05, $NaHCO_3$ 11·90, dextrose 5·55. Tension was recorded using Grass force–displacement isometric transducers (FT03) and a Grass polygraph (79E). The tissue was equilibrated for 45–60 min under 1 g resting tension before drugs were administered.

Concentration–response curves were generated by adding acetylcholine cumulatively (Van Rossum 1963) at 30–45 s intervals to the bath. An initial concentration–response curve was obtained and defined as the control. The ileum was then incubated for 15 min with ADT 16 or vehicle and a second concentration–response curve to acetylcholine obtained. Subsequent concentration–response curves in the presence of higher concentrations of ADT 16 were obtained after washing the ileum and restoration of the baseline tension. The mean contractile response to each concentration of acetylcholine, in the control period and in the presence of various concentrations of ADT 16, was expressed as a percentage of the control mean maximum response.

Time control studies. Parallel experiments in which the tissues described above were not exposed to any antagonist were performed, to determine any time-dependent changes in agonist sensitivity.

Actions on the CNS

Flexor reflex activity (FRA) in the spinal and decerebrate rat. The method used was a modification of that described by Maj et al (1976) as previously described by Rawlow & King (1991). Male Wistar rats, 250–300 g, were anaesthetized with halothane (4% halothane in 1 : 1 N_2/O_2), spinalized at T10 by transecting the spinal cord with scissors, tracheotomized and decerebrated with a pithing rod down to C1. Each rat was ventilated at a rate of 50 strokes min^{-1}, 3 mL $stroke^{-1}$. Blood

pressure was monitored from the right carotid artery with a pressure transducer connected to a Grass recorder (model 79B). The left carotid was ligated.

The tendon of the right anterior tibialis muscle was exteriorized and attached to an isometric transducer under a resting tension of 1 g. Reflex twitches of the muscle were evoked by electrical impulses (15 V, 20 ms, 0·1 Hz) delivered transcutaneously by a pair of needle electrodes to the ipsilateral hind paw (Grass S88 stimulator). Twitches were recorded on a Grass polygraph (model 79D). Rectal temperature was maintained at 36–37°C by placing the rat on a heated table.

Baseline FRA was defined as the mean tension developed in the 8·5–10 min after beginning the stimulation. Fenfluramine (2 mg kg^{-1}, i.v.) or clonidine (0·5 mg kg^{-1}, s.c.) was administered 10 min after stimulation was started to cause facilitation of the FRA. Fenfluramine-facilitated FRA and clonidine-facilitated FRA was defined as the mean increase in FRA in the 18·5–20 or 58·5–60 min after administration of fenfluramine or clonidine, respectively. ADT 16 (1, 3 and 10 mg kg^{-1}, i.v.) or mianserin (0·3, 1 and 3 mg kg^{-1}, i.v.) was injected in a series of cumulative doses at 10-min intervals, the first injection being made 20 or 60 min after fenfluramine or clonidine, respectively. All compounds were injected into the tail vein in a volume of 1 mL kg^{-1} over 1 min.

FRA was measured 8·5–10 min after each injection of ADT 16, mianserin or saline and compared using paired Student's *t*-test.

The myoclonic twitch test in the anaesthetized rat. The method used was that described previously by Rawlow & King (1990). Male Wistar rats, 250–350 g, were anaesthetized with urethane (1·2–1·8 g kg^{-1}, i.p.), and the trachea was cannulated. The right anterior digastricus muscle was freed from its attachment to the right posterior digastricus muscle, and attached to an isometric transducer (Grass force displacement transducer FT03) under a resting tension of 2 g. The left carotid was cannulated to monitor blood pressure and heart rate (Gould Statham P23 pressure transducer) and the jugular vein cannulated to administer drugs. Twitches of the right anterior digastricus muscle, blood pressure and heart rate were all recorded as described above simultaneously on a Grass Model 79D polygraph. Rectal temperature was maintained at 36·5–37°C by placing the rat on a heated table.

In experiments with L-5-hydroxytryptamine (L-5-HTP) (100 mg kg^{-1}, i.v.) and L-3,4,-dihydroxyphenylalanine (L-dopa) (100 mg kg^{-1}, i.p.), animals were pretreated intraperitoneally either with the peripheral aromatic amino acid decarboxylase inhibitor carbidopa (25 mg kg^{-1}, 30 min before L-5-HTP), or with the monoamine oxidase inhibitor nialamide (50 mg kg^{-1}) and carbidopa (25 mg kg^{-1}) 120 and 30 min before L-dopa, respectively. Saline (1 mL kg^{-1}, i.v., three consecutive doses) or ADT 16 (1 and 3 mg kg^{-1}, i.v.) was injected (the latter as cumulative doses) at 10-min intervals, the first dose being injected 10 or 40 min after L-5-HTP or L-dopa, respectively. The frequency of twitches was measured during the 5–10 min interval after each injection of saline or ADT 16 and expressed as twitches min^{-1}.

Drugs

ADT 16 was prepared as described above (Browne 1985). Other compounds used were acetylcholine chloride, clonidine hydrochloride, L-3,4-dihydroxyphenylalanine methyl ester, 5-hydroxytryptamine creatinine sulphate, L-5-hydroxytryptophan, nialamide, phenylephrine hydrochloride (all from Sigma Chemical Co., USA), B-HT 920 dihydrochloride (Boehringer Ingelheim, Germany), carbidopa (Merck Sharp and Dohme, Australia), fenfluramine (Riker Laboratories, Australia), halothane (ICI, Australia), mianserin hydrochloride (Research Biochemical Inc., USA), urethane (ethyl carbamate, Ajax Chemicals, Australia), dimethylsulphoxide (DMSO) (BDH Chemicals, Australia). Doses of drugs used refer to their salts whenever applicable.

ADT 16 was dissolved in a (5:2:3) mixture of DMSO, ethanol (70%) and distilled water and diluted with saline (0·9% NaCl, w/v). All other compounds were dissolved in saline except for phenylephrine which was dissolved in catecholamine diluent (0·156 g NaH_2PO_4 and 0·04 g ascorbic acid L^{-1} saline) and nialamide which was dissolved in distilled water with addition of two to three drops of 0·1 M HCl L^{-1}.

For the in-vivo studies, nialamide, carbidopa or L-dopa was injected intraperitoneally in a volume of 4 mL kg^{-1}. All other drugs (except clonidine which was injected subcutaneously) were injected intravenously in a volume of 1 mL kg^{-1} over 1 or 3 min (ADT 16, 3 and 10 mg kg^{-1}).

Statistical analysis

Data were expressed as mean ± s.e.m., and where appropriate, Student's paired or unpaired *t*-tests were used for comparisons between means. Statistical significance was accepted for $P < 0.05$. Log concentration–response curves were analysed by regression analysis over the linear portion of the curves (Diem & Leutner 1970). Concentration-ratios were determined from the concentrations causing 50% of the maximum response (EC50) in the absence or presence of an antagonist and the data used to generate a Schild plot (Arunlakshana & Schild 1959).

Results

In-vitro studies

Effects of ADT 16 and mianserin on the inhibitory effects of B-HT 920 on rat isolated transmurally stimulated vas deferens. As shown in Fig. 2a, ADT 16 (100 and 1000 nM, 15 min contact) shifted the B-HT 920 log concentration–response curve to the right. ADT 16 was calculated to have an apparent pA_2 value of 6·22 ± 0·05. However, the slope of the Arunlakshana–Schild plot was significantly different from unity (1·26 ± 0·09; n = 3–5). Mianserin also shifted the log concentration–response curve to the right (Fig. 2b) with a calculated pA_2 value of 6·77 ± 0·14, slope 0·97 ± 0·16; n = 5, not significantly different from 1·0.

In the absence of B-HT 920, high concentrations (10 μM) of ADT 16 (n = 3) or mianserin (n = 5) significantly potentiated ($P < 0.05$) the tension of the twitch responses to electrical stimulation to 213 ± 34 or 198 ± 12% of the control twitch responses, respectively.

Effects of ADT 16 on contractile responses of rat isolated epididymal vas deferens to phenylephrine. ADT 16 (0·3–1 μM,

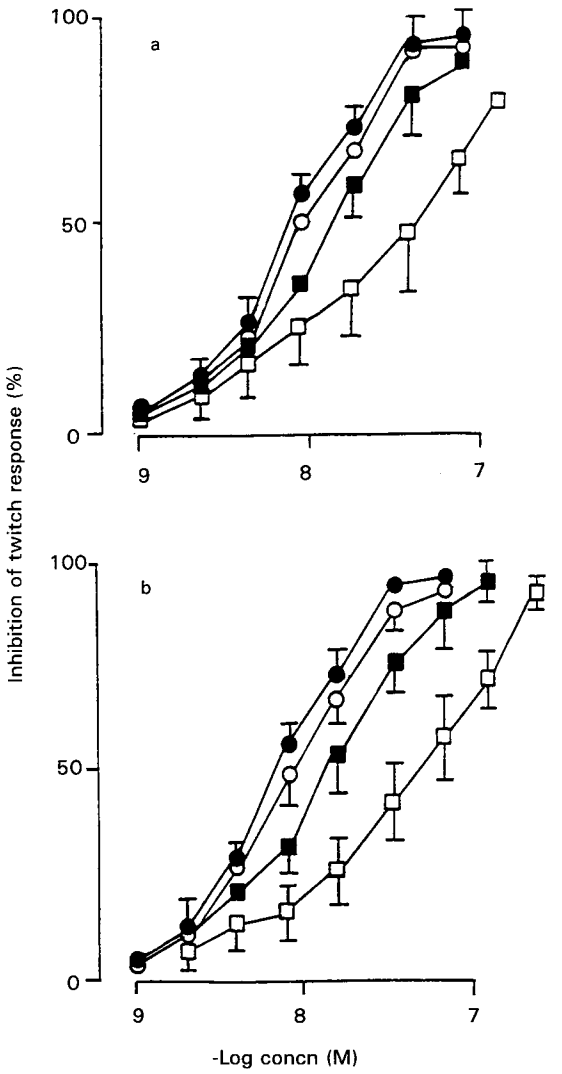

FIG. 2. Effects of ADT 16 or mianserin on inhibition caused by B-HT 920 of the electrically stimulated rat vas deferens. Control B-HT 920 curve (●), responses after (a) ADT 16 or (b) mianserin; ○ 10, ■ 100 or □ 1000 nM. Vertical lines s.e.m., n = 3–5.

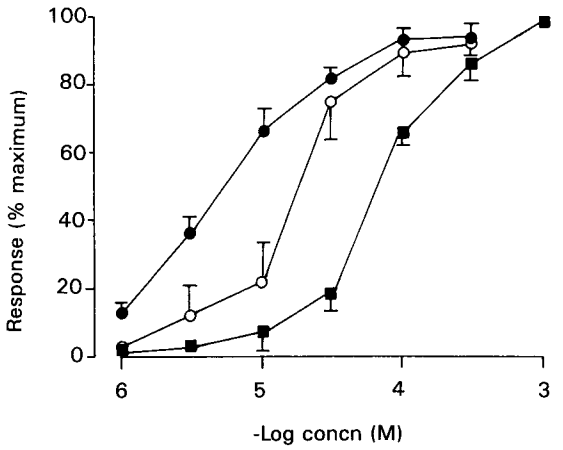

FIG. 3. Effects of ADT 16 on responses to phenylephrine of rat epididymal vas deferens. Control phenylephrine curve (●), responses after ADT 16: ○ 0·3 and ■ 1 μM. Points are mean and vertical lines s.e.m., n = 3–5.

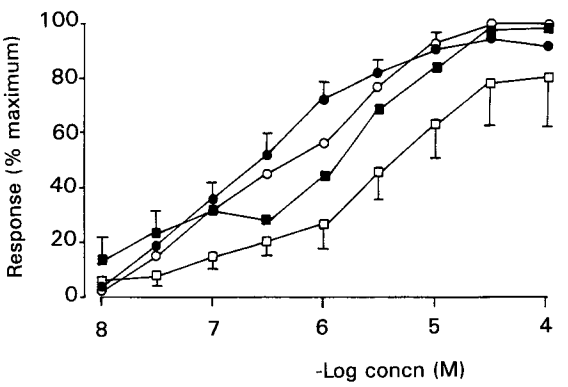

FIG. 4. Effects of ADT 16 on responses to 5-HT of rat stomach strip. Control 5-HT curve (●), responses after ADT 16: ○ 0·3, ■ 1 and □ 3 μM. Points are mean and vertical lines s.e.m., n = 4–5.

15 min contact) shifted the phenylephrine log concentration–response curve to the right (Fig. 3). ADT 16 was calculated to have an apparent pA_2 value of 6·77 ± 0·7. However, the slope of the Schild plot was significantly different from unity ($P < 0·05$, i.e. 1·25 ± 0·08; n = 3–5). The corresponding vehicle of ADT 16 (DMSO, ethanol, distilled water) had no effect on responses to phenylephrine (data not shown, n = 3–5).

Effects of ADT 16 on responses to 5-HT of rat isolated stomach fundus strips. As shown in Fig. 4, ADT 16 (1–3 μM, 15 min contact) shifted the 5-HT log concentration–response curve to the right. ADT 16 was calculated to have an apparent pA_2 of 6·3 ± 1·3. However, the slope of the Schild plot was significantly different from unity ($P < 0·05$, i.e. 1·7 ± 0·4). The corresponding vehicle of ADT 16 (DMSO, ethanol, distilled water) had no effect on responses to 5-HT (data not shown, n = 3–5).

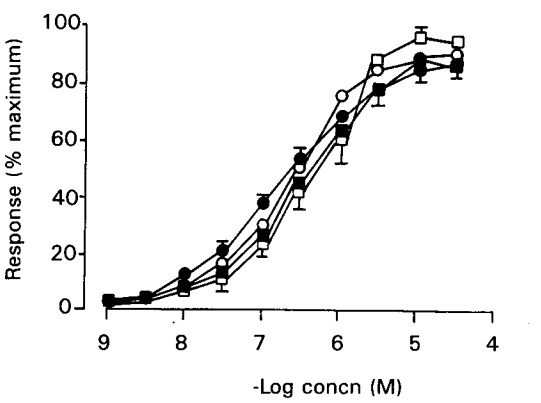

FIG. 5. Effects of ADT 16 or vehicle on responses to acetylcholine of guinea-pig ileum. Control acetylcholine curve (●), responses following vehicle (□) and ADT 16: ○ 0·1 and ■ 1 μM. Points are mean and vertical lines are s.e.m., n = 4.

Table 1. The effect of ADT 16 on flexor reflex tension facilitated by fenfluramine (2 mg kg^{-1}, i.v.) or clonidine (0·5 mg kg^{-1}, s.c.).

	Increase in flexor reflex tension (g)			
	Fenfluramine (n = 3)		Clonidine (n = 2–5)	
ADT 16	Control	ADT 16	Control	ADT 16
1	4·8 ± 1·7	4·2 ± 1·8	15·2 ± 0·8	11·2 ± 2·4
3	4·5 ± 1·1	1·5 ± 0·9*	14·8 ± 0·7	2·2 ± 1·2
10	5·0 ± 0·9	0·5 ± 0·1**	—	—

* $P < 0·05$, ** $P < 0·01$ compared with corresponding control.

Table 2. The effect of intravenous ADT 16 on the frequency of twitches of the anterior digastricus muscle evoked by L-dopa (100 mg kg^{-1}, i.p.) or L-5-HTP (100 mg kg^{-1}, i.v.) in the anaesthetized rat (n = 4).

	Myoclonic twitches (twitches min^{-1}) evoked by	
ADT 16 (mg kg^{-1})	L-dopa	L-5-HTP
0	2·4 ± 0·3	2·6 ± 0·2
1	1·2 ± 0·4*	1·3 ± 0·2*
3	0·4 ± 0·4*	0·5 ± 0·1*

*Effect of ADT 16 significantly different from corresponding control ($P < 0·05$).

Effects of ADT 16 on responses of the guinea-pig ileum to acetylcholine. As shown in Fig. 5, ADT 16 (0·1 and 1 μM) had no effect on the concentration–response curves for the contractile effect of acetylcholine on guinea-pig isolated ileum.

Time control studies. In all four isolated tissue preparations used, there were no significant time-dependent changes of the agonist concentration–response curves (n = 4, data not shown).

Actions on the CNS

Flexor reflex activity in the rat. ADT 16 (3 and 10 mg kg^{-1}, i.v.) significantly attenuated fenfluramine-facilitated FRA in the rat in a dose-dependent manner ($P < 0·05$; Table 1). At the lower dose of 1 mg kg^{-1} (i.v.), ADT 16 had no effect on fenfluramine-facilitated FRA in the rat.

ADT 16 (3 mg kg^{-1}, i.v., n = 2) also reduced FRA facilitated by clonidine (Table 1). However, 1 mg kg^{-1} (n = 5) had no effect.

The myoclonic twitch test in the anaesthetized rat. There were no spontaneous twitches of the right anterior digastricus muscle in the 10-min period preceding administration of L-5-HTP or L-dopa. Administration of L-dopa to nialamide- and carbidopa-pretreated rats caused the appearance of myoclonic twitches of the muscle, which reached a maximum frequency 40 min later. The frequency of twitches remained constant over the next 80 min in animals injected with saline (data not shown, n = 4). ADT 16 (1 and 3 mg kg^{-1}, i.v.) significantly reduced the frequency of twitches of the muscle evoked by L-dopa (100 mg kg^{-1}, i.p.) (Table 2).

Following administration of L-5-HTP to carbidopa-pretreated rats, myoclonic twitches of the muscle occurred, reaching a maximum frequency 30–40 min later. The frequency of twitches remained constant over the next 40–50 min (data not shown, n = 4). ADT 16 (1 and 3 mg kg^{-1}, i.v.; n = 4) significantly attenuated the frequency of the twitches of the anterior digastricus muscle evoked by L-5-HTP (100 mg kg^{-1}, i.v.) (Table 2).

Discussion

The aim of these studies was to determine some of the acute peripheral and central actions of ADT16, a novel heterocyclic compound, on responses mediated by 5-HT-ergic and adrenergic neurons of the rat. These results demonstrate that ADT 16 resembles the tetracyclic antidepressant mianserin, as it was found to inhibit both peripheral and central α-adrenoceptor- and 5-HT-mediated responses in the rat.

Using the rat isolated vas deferens preparation, ADT 16 displayed similar pharmacological properties to mianserin. This tissue has inhibitory prejunctional α_2-adrenoceptors along its length (Pennefather et al 1974). The effects of α_2-adrenoceptor agonists are greatest at the prostatic end of the vas deferens, since the density of α_1-adrenoceptors (which can mask the effects of α_2-adrenoceptor agonists) is lower (Pennefather et al 1974; Vardolov & Pennefather 1976; Kasuya & Suzuki 1979; Brown et al 1979). B-HT 920 is a potent and selective α_2-adrenoceptor agonist (Kobinger & Pichler 1981) causing concentration-dependent inhibition of the electrically-stimulated twitch responses of the rat isolated prostatic vas deferens due to activation of presynaptic adrenergic nerve terminal α_2-adrenoceptors. ADT 16 similarly to mianserin caused rightward shifts of the B-HT 920 log concentration response and potentiation of responses to electrical stimulation after high concentrations (in the absence of B-HT 920). These results indicate that in the rat, both compounds have presynaptic α_2-adrenoceptor antagonist properties in-vitro. Mianserin has previously been shown to act as an antagonist at these receptors in the same species (Brown et al 1980). ADT 16 also antagonized phenylephrine-induced contractions of the rat isolated epididymal vas deferens. Phenylephrine is a selective α_1-adrenoceptor agonist (Ruffolo 1983) which causes concentration-dependent increases of the contractile responses of this preparation. Mianserin has been reported to cause weak noncompetitive antagonism of noradrenaline-induced contractions of rat isolated vas deferens (postsynaptic α_1-adrenoceptors) (Van Riezen et al 1981).

In the present study, ADT 16 was an antagonist of 5-HT in the rat fundus strip. Mianserin has been shown to display noncompetitive antagonism of 5-HT-induced contractions of the rat stomach fundus (Cohen & Fludzinski 1987; Leitch et al 1992b). The contractile effect of 5-HT on the rat stomach strip is thought to be mediated by 5-HT_{2C} receptors (Humphrey et al 1993), previously known as 5-HT_{1C} receptors (Buchheit et al 1986).

ADT 16 did not affect acetylcholine-induced contractions of the ileum, indicating that it has little effect on muscarinic cholinoceptors. Mianserin has been previously reported (Vargaftig et al 1971) not to antagonize acetylcholine-induced contractions of the guinea-pig ileum and is also

known to be devoid of anticholinergic activity in-vivo (Van Riezen et al 1981).

In-vivo, ADT 16 (3–10 mg kg^{-1}, i.v.) attenuated fenfluramine-induced facilitation of the flexor reflex in rats, an effect mediated by spinal tryptaminergic mechanisms (Maj et al 1976). Mianserin has previously been shown (Leitch et al 1992a) to be a potent antagonist (ID50 0·36 mg kg^{-1}, i.p.) of fenfluramine-induced facilitation of FRA in the spinalized and decerebrate rat.

ADT 16 also attenuated the facilitation of FRA induced by clonidine, suggesting that it is a centrally acting α_2-adrenoceptor antagonist. These results in-vivo support data from the rat vas deferens preparation indicating that ADT 16 is an α_2-adrenoceptor antagonist. Other compounds with some structural similarity to ADT 16 have been shown to act as α_2-adrenoceptor antagonists (Chapleo 1988). The α_2-adrenoceptor agonist, clonidine facilitates FRA in the spinalized and decerebrate rat due to activation of central postsynaptic α_2-adrenoceptors (Rawlow & Gorka 1986). Mianserin has previously been shown (Leitch et al 1992a) to be a potent antagonist (ID50 0·48 mg kg^{-1}, i.p.) of clonidine-induced facilitation of FRA in the spinalized and decerebrate rat.

In the rat myoclonic twitch test, L-dopa or L-5-HTP evoke spontaneous twitches of the anterior digastricus muscle which are attenuated by α_1-adrenoceptor antagonists or 5-HT-receptor antagonists, respectively (Rawlow & King 1990). ADT 16 reduced the frequency of twitches evoked by L-dopa or L-5-HTP indicating that it is a centrally-active compound affecting adrenergic and 5-HT systems in the rat. In-vivo, the antagonistic effects of ADT 16 lasted less than 30 min.

In summary, it is concluded that ADT 16 is a potent peripherally- and centrally-active novel heterocyclic compound which penetrates the blood-brain barrier and which inhibits responses mediated by 5-HT and adrenergic neurons or receptors in the rat.

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