Naunyn-Schmiedeberg's Arch Pharmacol (1984) 326:36–44

Naunyn-Schmiedeberg's
Archives of
Pharmacology

MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors

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Summary. The properties of MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate), a novel compound with potent and selective blocking actions at certain excitatory 5-hydroxytryptamine (5-HT) receptors on mammalian peripheral neurones, are described.

On the rabbit isolated heart, MDL 72222 was a potent antagonist of responses mediated through the receptors for 5-HT present on the terminal sympathetic fibres. The threshold for antagonism was approximately 0.1 nM and the negative logarithm of the molar concentration of MDL 72222 which reduced the chronotropic response of the isolated rabbit heart to twice an ED_{50} of 5-HT to that of the ED_{50} was 9.27. MDL 72222 was also highly selective since responses to the nicotine receptor agonist, dimethylphenylpiperazinum iodine (DMPP), were inhibited only at concentrations more than 1000 times those necessary to inhibit 5-HT.

In the anaesthetised rat, MDL 72222 produced marked blockade of the Bezold-Jarisch effect of 5-HT. Again, inhibition was selective since much higher doses of MDL 72222 failed to alter the response to electrical stimulation of the efferent vagus nerves. In contrast, MDL 72222 proved only a weak and essentially non-selective antagonist of responses mediated by the 5-HT M-receptor present on the cholinergic nerves of the guinea-pig ileum.

MDL 72222 does not block smooth muscle contractile responses elicited by oxytocin or mediated through 5-HT D-receptors, muscarinic or nicotinic cholinoceptors or histamine H_1-receptors except at relatively high concentrations. Similarly, in a number of radioligand binding assays carried out using brain tissue membranes, the displacing effects of MDL 72222 were absent or weak at sites identifying compounds with activity at α_1, α_2 or β-adrenoceptors, 5-HT_1 or 5-HT_2 receptors, benzodiazepine receptors or histamine H_1-receptors.

MDL 72222 is the first reported selective and potent antagonist of responses mediated through the 5-HT receptors present on the terminal sympathetic neurones of the rabbit heart and on the neurones subserving the afferent limb of the Bezold-Jarisch reflex. The compound should provide a useful means by which responses mediated through such sites can be distinguished.

Key words: 5-HT receptors – Autonomic neurones – Sensory neurones – MDL 72222 – Transmitter release

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Introduction

5-Hydroxytryptamine (5-HT) excites a variety of neurones in the mammalian peripheral nervous system through activation of neuronal 5-HT receptors (Wallis 1981). Morphine is not a selective antagonist at these receptors although cocaine and a number of its analogues (Fozard 1979; Fozard et al. 1979) and metoclopramide (Fozard and Mobarok Ali 1978a) show both limited potency and a degree of selectivity at certain of these sites both in vitro and in vivo (Fozard and Host 1982). In seeking to optimize such activity, the neuronal 5-HT receptor blocking properties of a series of substituted benzoic acid esters of tropine were investigated (preliminary details in Fozard and Gittos 1983). In the present report, I describe the properties of one of these compounds, 1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222; formula in Fig. 1) which proved to be both potent and highly selective as an antagonist at excitatory 5-HT neuronal receptors. The interaction of MDL 72222 with 5-HT has been investigated on the rabbit isolated heart, where stimulation of 5-HT receptors results in noradrenaline release from the terminal sympathetic fibres (Fozard and Mwaluko 1976; Fozard and Mobarok Ali 1978b; Göthert and Dührsen 1979), on the guinea-pig ileum, where excitatory 5-HT receptors mediate acetylcholine release from the intramural cholinergic nerves (Gaddum and Picarelli 1957), and in the anaesthetized rat, where activation of receptors on vagal afferent fibres initiates the Bezold-Jarisch effect of 5-HT (Salmoiraghi et al. 1956; Krayer 1961; Paintal 1973; Fozard and Host 1982).

Preliminary reports of these findings have been presented to the British Pharmacological Society (Fozard 1983a; Fozard and Gittos 1983).

Methods

Techniques to measure neuronal 5-HT receptor blocking activity rabbit heart. Rabbits of either sex weighing 2.1–2.9 kg were given heparin (500 U/kg) into a marginal ear vein. Two to 5 min later they were stunned by a blow to the head and bled. Hearts were rapidly removed and perfused according to the Langendorff technique with modified Tyrode's solution at 35.5°C. The Tyrode's solution (concentrations in mmol/l: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, glucose 5.6 and ascorbic acid 0.06) was gassed with a mixture of 95% O_2 and 5% CO_2. Perfusion pressure was maintained at approximately

60 cm water. Atropine, 1.7 μmol/l, was added routinely to the perfusion fluid to prevent interference from indirect muscarinic activity (Fozard and Muscholl 1971). Right ventricular tension and rate and right atrial tension were recorded as described by Fozard and Muscholl (1971). Dose-effect curves to 5-HT and dimethylphenylpiperazinium iodide (DMPP) on atrial and ventricular tension development and ventricular rate were established using bolus injections delivered manually in under 1 s by microlitre syringe. The volume of injection was kept below 100 μl and injection of this volume of ice cold saline had no effect on cardiac performance. Usually the interval between doses was 5 min but if the effects of a previous dose were still in evidence, this interval was extended. In control experiments, up to four successive dose-response curves were established with a 15 min interval between each. The effects of MDL 72222 were assessed by including it in the perfusion solution in increasing concentrations prior to the subsequent curves and comparing the changes produced with the control situation where no modifying drug was present.

To quantify antagonism, a method based on that described by Schild (1947) for the determination of pA_2 values was adopted using cardiac rate as the quantified response. Thus, the molar concentration of antagonist was determined which reduced the effects of twice the ED_{50} of agonist to that of the ED_{50} in the absence of antagonist. This was expressed as a negative logarithm. The values so obtained afford a convenient measure of antagonism. They do not imply any particular mechanism of agonist/antagonist interaction.

Guinea-pig ileum. Segments of ileum were removed from freshly killed guinea-pigs and set up for recording changes in longitudinal tension in the modified Tyrode's solution described above. Methysergide, 2.8 μmol/l was included in the Tyrode's solution to exclude interference from the direct muscle stimulant responses to 5-HT (Fozard and Mobarok Ali 1978 b). The tissue was stimulated transmurally by means of platinum ring electrodes (ring diameter 8 mm, distance between rings 32 mm) and a Grass SD9 Square wave stimulator. Stimulation parameters were: 0.5 ms, supramaximal voltage, 0.5 – 16 Hz, 15 s.

Immediately after setting up, the preparations were stimulated with an approximate ED_{50} of either 5-HT, DMPP or acetylcholine, or a frequency of transmural nerve stimulation of 2 Hz, every 3 min until responses were reproducible. Thereafter, responses remained constant and up to 5 concentration/frequency response curves could be obtained at 15 min intervals with little change in sensitivity being apparent (Fozard et al. 1979). In the experiments with MDL 72222, the drug was introduced into the incubation solution immediately after the first curve had been obtained with subsequent curves being established in cumulatively increasing concentrations of the antagonist.

Blood pressure and heart rate in anaesthetized rats. In most experiments, male Spraque-Dawley rats (Charles River, France) weighing 260 – 380 g were anaesthetized with urethane, 1.25 g/kg i.p. Blood pressure was recorded from the left common carotid artery by means of a Statham pressure transducer (Type P23 AA) and heart rate was recorded from the electrocardiogram using a Beckman cardiotachometer coupler (Type 9757 B). Records were displayed on a Beckman dynograph (Type R). A femoral or jugular vein was cannulated for the intravenous injection of drugs. After completion of all operative procedures, heparin, 500 units, was injected intravenously. In some experiments, rats were lightly anaesthetized with pentobarbitone sodium, 40 mg/kg, i.p. and bilaterally adrenalectomized prior to pithing by the method of Shipley and Tilden (1947).

The Bezold-Jarisch effect of 5-HT was elicited in the anaesthetized preparations by the rapid bolus intravenous injection of 5-HT, 1.25 – 10 μg/kg. The initial abrupt cardiac slowing and associated fall in blood pressure result from reflex stimulation off the vagus following activation of sensory afferent fibres located mainly in the right ventricle (Salmoiraghi et al. 1956; Krayer 1961; Paintal 1973). An ID_{50} value for MDL 72222 was obtained by injecting the antagonist i.v. 5 min before challenge with 5-HT and the dose was derived which reduced a submaximal response to 5-HT (usually 2 μg/kg) by 50%. In some animals, the left vagus nerve was isolated, cut and the distal portion stimulated electrically. Stimulation parameters were: 2 – 20 Hz; 5 s; 1 ms; just supramaximal voltage (20 – 40 V).

Experiments with ^{3}H-noradrenaline. Rabbit hearts were perfused according to the Langendorff technique as described above except that flow rate was maintained constant at either 20 or 40 ml/min (see below). The neuronal noradrenaline stores were labelled with ^{3}H-noradrenaline by the procedure described previously (Fozard et al. 1979). Briefly, (–)-7-^{3}H-noradrenaline (sp. act. 14.8 Ci/mmol; 15% of label in position 8) was diluted freshly each day with unlabelled noradrenaline to give the stock solution. This was infused at the rate of 0.1 ml/min (with a Palmer slow injection apparatus) into the arterial inflow to give a final concentration in the medium of 59 nmol/l and radioactivity concentration of 50 nCi/ml. Flow rate was maintained at 20 ml/min by means of a roller pump (Watson-Marlowe Type MHRE 200) during the loading period (12 min) and increased to 40 ml/min thereafter.

In control experiments, test doses of 5-HT (11, 45, 182 and 727 nmol), tyramine (146 and 583 nmol) and DMPP (26, 105 and 418 nmol) were introduced by rapid bolus injections into the arterial inflow at times 30, 35, 40, 45, 55, 65, 75, 80, 85 min, respectively, after the end of the loading period with ^{3}H-noradrenaline. In test experiments, the sequence was repeated on hearts perfused from the end of the loading period with MDL 72222, 5 nmol/l. Samples of perfusate were collected at the intervals specified in "Results" and the total radioactivity was measured by dispersing a 1 ml aliquot in 10 ml scintillation fluid (Aquasol-2, NEN) and counting in a liquid scintillation spectrometer.

Other pharmacological preparations. The uterus, fundus and aorta of the rat were prepared for recording contractile responses by established techniques (University of Edinburgh Staff, 1970). The potassium-depolarized, guinea-pig taenia caeci preparation was set up according to the method of Spedding (1982). Local anaesthetic activity was measured by the guinea-pig wheal test of Bülbring and Wajda (1945).

Radioligand binding assays. Standard techniques were used to identify radioligand binding to brain membranes (Leysen et al. 1981). Further details of the tissues and radiolabelled ligands used are presented in Table 2.

Statistical analysis

All measures of mean variation quoted are standard errors. Student's *t*-test was used to assess the significance of differences between mean values. The number of observations is indicated by *n*.

Drugs

Drugs obtained from commercial sources were acetylcholine chloride (Sigma, St. Louis, MO, USA), atropine sulphate (Meram, Paris, France), dimethylphenylpiperazinium iodide (Aldrich, Chemical Co., Amersham, Buckinghamshire, GB), heparin (Choay, Paris, France), histamine dihydrochloride (Sigma, St. Louis, MO, USA), 5-hydroxytryptamine creatinine sulphate (Sigma, St. Louis, MO, USA), lignocaine hydrochloride (Astra, Mölndal, Sweden), methysergide bimaleate (Sandoz, Basel, Switzerland), oxytocin, tyramine hydrochloride (Sigma, St. Louis, MO, USA). 4-(m-Chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) was a gift from McNeil Labs. Inc., Pittsburg, PA, USA. MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate) was synthesized in the Department of Chemistry, Centre de Recherche Merrell International, Strasbourg by Dr. M. Gittos. (−)-7-^{3}H-Noradrenaline, and all radiolabelled ligands reported in Results, Table 2, were obtained from New England Nuclear, Dreieich, FRG.

Results

Effects of MDL 72222 on chronotropic responses evoked by 5-HT and DMPP on the rabbit heart

Bolus injections of 5-HT (5.7 – 364 nmol) or DMPP (52 – 418 nmol) gave dose-related increases in the force (data not illustrated) and rate of beating of the isolated rabbit heart (see control curves in Fig. 1) which were reproducible when re-established up to three times with a 15 min interval between each curve (data not illustrated). The maximum increases in cardiac rate were close to 100 beats/min and similar for both 5-HT and DMPP.

The results of including MDL 72222 in the perfusion fluid 15 min prior to establishing the dose-response curves to 5-HT or DMPP are shown in Fig. 1. Concentrations of MDL 72222, between 0.32 and 6.36 nmol/l, produced concentration-dependent inhibition of the chronotropic response to 5-HT (Fig. 1). At the the lowest concentration, the curve was shifted in parallel to the right and there was no depression of the maximum response; at the higher concentrations both the slopes and the maxima of the 5-HT log dose response curves were progressively decreased (Fig. 1 A). Much higher concentrations of MDL 72222 were required to inhibit chronotropic responses to DMPP than were effective against 5-HT (compare Fig. 1 A and B). At 0.64 μmol/l the log dose-response curve to DMPP was shifted in parallel to the right; whith a 2.5-fold higher concentration there was further inhibition but both the slope and the maximum of the log dose-response curve were decreased (Fig. 1 B).

In separate experiments, the negative logarithm of the molar concentration of MDL 72222 which reduced the chronotropic response of the isolated rabbit heart to twice an ED_{50} of 5-HT to that of the ED_{50} was estimated (see Methods) and found to be 9.27 ± 0.06 ($n = 7$). The equivalent value with DMPP as the agonist was 6.14 ± 0.04 ($n = 6$). Thus, MDL 72222 is approximately 1,000 times more selective as an inhibitor of responses mediated through neuronal 5-HT receptors.

The effect of MDL 72222 on the overflow of radioactivity evoked by 5-HT, tyramine and DMPP from rabbit hearts loaded with ^{3}H-noradrenaline

As previously described (Fozard 1979; Fozard et al. 1979), 5-HT (11 – 727 nmol), tyramine (146 and 583 nmol) and DMPP (26 – 418 nmol) evoked dose-related increases in the outflow of radioactivity from hearts whose transmitter stores were labelled with ^{3}H-noradrenaline (Fig. 2). Perfusion with MDL 72222 (5 nmol/l) from the end of the loading period caused no significant change in the spontaneous outflow of radioactivity but markedly inhibited both the cardiac stimulant responses (data not illustrated) and the outflow of radioactivity evoked by 5-HT (Fig. 2). MDL 72222 did not alter significantly either the overflow of radioactivity (Fig. 2) or the cardiac stimulant responses (data not illustrated) evoked by tyramine or DMPP.

Effects of MDL 72222 on the Bezold-Jarisch effect of 5-HT in the anaesthetized rat

In the rat anaesthetized with urethane, rapid bolus injections of 5-HT (1.25 – 10 μg/kg) elicit abrupt, dose-related falls in cardiac rate of short duration. Blood pressure initially falls, associated with the fall in cardiac rate, then, in most instances, rises transiently before showing a relatively sustained (2 – 5 min) fall with no associated change in heart rate

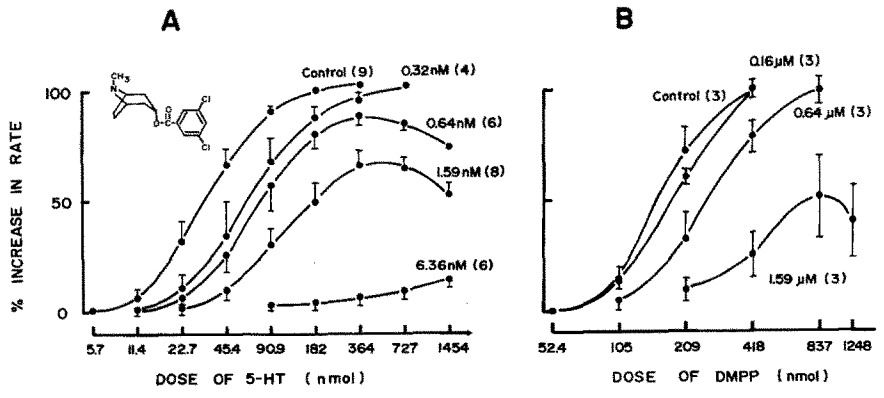

Fig. 1
The effects of MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate; structure shown in part **A** on the changes in rate of rabbit isolated hearts evoked by bolus injections of 5-HT (**A**) and DMPP (**B**). Dose-response curves were established (control) and repeated after 15 min perfusion with MDL 72222 at the concentrations shown. The *points* represent the means with standard errors of the numbers of individual determinations shown in parentheses. Note that nanomolar concentrations of MDL 72222 are effective against 5-HT whereas micromolar concentrations are needed to inhibit DMPP

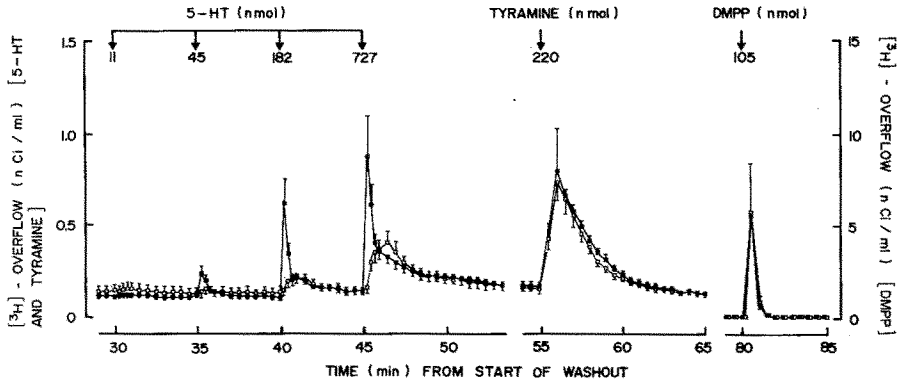

Fig. 2. Spontaneous and evoked outflow of radioactivity from rabbit isolated hearts whose neuronal transmitter stores had been labelled by perfusion with ^{3}H-(−)-noradrenaline (59 nmol/l; 50 nCi/ml) for 12 min. Bolus injections of 5-HT, tyramine and DMPP were given at the times and doses indicated to hearts perfused throughout with Tyrode (■) or to hearts perfused from the end of the loading period (start of washout) with Tyrode containing MDL 72222, 5 nmol/l (□). The *points* represent the means of 4–5 individual observations; *vertical lines* are standard errors of the mean values. For clarity of presentation, only the results obtained with the lower dose of tyramine and the intermediate dose of DMPP (see Methods) are illustrated

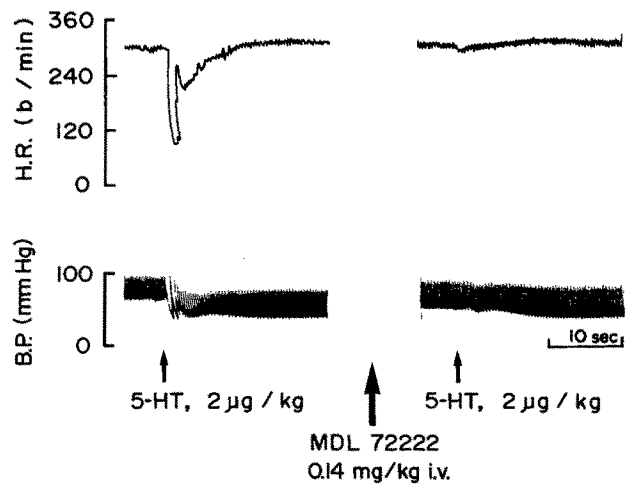

Fig. 3. Selective blockade of the Bezold-Jarisch component of the cardiovascular response to 5-HT in the anaesthetized rat. The second 5-HT injection was given 8 min after the first and 3 min after MDL 72222

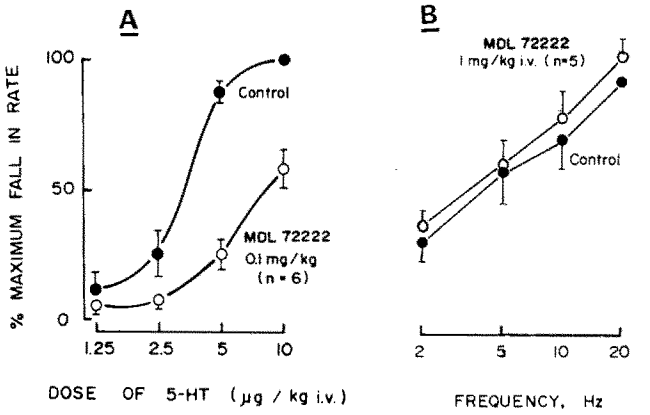

Fig. 4A, B. Selective blockade of the Bezold-Jarisch effect of 5-HT in the anaesthetized rat by MDL 72222. **A** Responses to 5-HT; **B** responses to electrical stimulation of the efferent vagus. ● Control responses; ○ responses repeated after intravenous administration of MDL 72222 at the doses shown. The control curves were reproducible under the conditions of the experiment. *Points* represent the mean values with standard errors of the number of individual determinations shown in parentheses

(illustrated for 5-HT, 2 µg/kg, in Fig. 3). Low doses of MDL 72222 produced marked attenuation of the Bezold-Jarisch component of the cardiovascular response to 5-HT (Figs. 3 and 4A). The ID_{50} for this effect measured against a submaximal dose of 5-HT (usually 2 µg/kg) was 0.039 ± 0.007 mg/kg ($n = 4$), and inhibition was selective, since doses of MDL 72222 as high as 1 mg/kg failed to alter the response to electrical stimulation of the efferent vagus nerves (Fig. 4B). A further indication of the selectivity of action of MDL 72222 is evident from Fig. 3 (and quantified elsewhere — Fozard and Host 1982), where a dose which abolished the Bezold-Jarisch component of the response to 5-HT had minimal effects on the secondary fall in blood pressure which is the dominant feature of the cardiovascular response to 5-HT in this species (Salmoiraghi et al. 1956; Fozard and Leach 1968).

A comparison of the potency and the duration of action of MDL 72222 as an inhibitor of the Bezold-Jarisch effect of 5-HT when given by the intravenous and intraduodenal routes of administration is shown in Fig. 5. When tested against a single, submaximal dose of 5-HT (usually 2 µg/kg) injected intravenously every 5 or 10 min in the anaesthetized rat, MDL 72222, 0.14 mg/kg, also given intravenously, caused rapid, marked but transient inhibition of the Bezold-Jarisch effect of 5-HT. Doubling the dose of MDL 72222 resulted in a marked increase in the duration of the inhibitory response (Fig. 5A). When given intraduodenally to the anaesthetized rat, MDL 72222 inhibited the Bezold-Jarisch component of the response to 5-HT at doses of 0.25, 0.5 and 1 mg/kg (Fig. 5B). Following a dose of 4 mg/kg, the response was rapidly suppressed and remained inhibited for the duration of the experiment (c. 85 min).

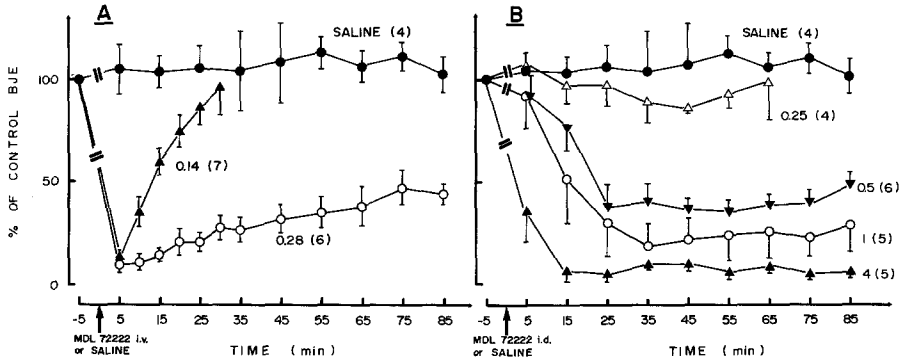

Fig. 5
Potency and duration of action of MDL 72222 given intravenously (**A**) or intraduodenally (**B**) as an inhibitor of the Bezold-Jarisch effect of 5-HT in the anaesthetized rat. A single submaximal dose of 5-HT (usually 2 μg/kg) was injected every 5–10 min. Saline or MDL 72222 at the doses shown (mg/kg) were administered as indicated. *Points* represent the mean values with standard errors of the number of individual observations shown in parentheses

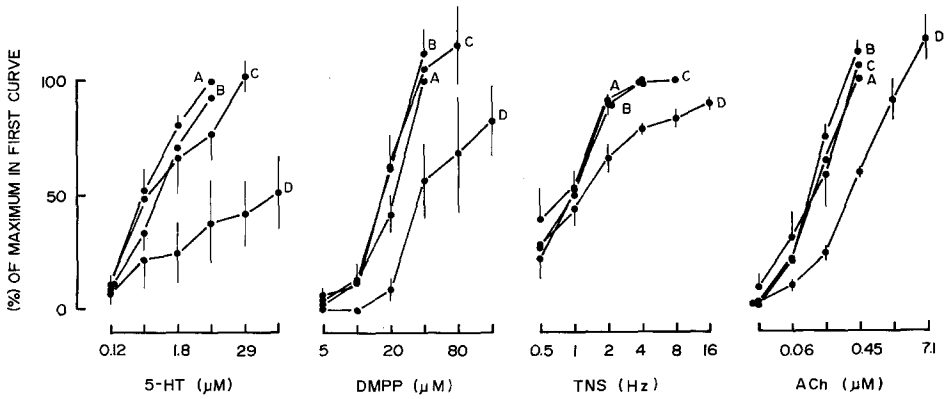

Fig. 6
Effects of MDL 72222 on responses of guinea-pig ileum to 5-HT, DMPP, transmural stimulation of the intramural cholinergic nerves (TNS) and acetylcholine (ACh). *A*, first (control) curve; *B*, *C* and *D* curves obtained in the presence of 0.32, 1.27 and 5.09 μmol/l of MDL 72222 respectively. *Points* represent the mean values with standard errors of 4–6 individual experiments. The control curves were reproducible and the conditions of the experiment

Effects of MDL 72222 on the responses of guinea-pig ileum to 5-HT, DMPP, transmural nerve stimulation and acetylcholine

The responses of the guinea-pig ileum to 5-HT in the presence of methysergide are exclusively indirect, the result of acetylcholine release from the intramural postganglionic cholinergic neurones (Fozard and Mobarok Ali 1978b). In contrast to its effects on the heart, MDL 72222 proved only a weak antagonist of responses to 5-HT mediated through the neuronal receptors of the ileum (compare Figs. 6 and 1A). Moreover, unlike in the heart, inhibition was not manifested selectively against 5-HT; responses to DMPP transmural nerve stimulation and added acetylcholine were all inhibited to approximately the same extent as those to 5-HT by MDL 72222, 5.09 μmol/l (Fig. 6).

Selectivity of action of MDL 72222

The effects of MDL 72222 against a number of agonists on a variety of tissues are summarized in Table 1. The compound has no selective blocking activity against smooth muscle contractile responses evoked by 5-HT in rat uterus, aorta and fundus. Similarly, MDL 722222 does not block responses elicited by oxytocin or mediated through muscarinic or nicotinic cholinoceptors or histamine H_1-receptors except at relatively high concentrations; the compound has no meaningful calcium antagonist activity measured as the capacity to inhibit calcium-induced contraction in potassium-depolarized taenia caeci (Table 1).

In a number of standard radioligand binding assays on brain tissue membranes, the displacing effects of MDL 72222 were absent or weak at sites identifying compounds with activity at α_1, α_2 or β-adrenoceptors, 5-HT_1 or 5-HT_2 receptors, benzodiazepine receptors or histamine H_1-receptors (Table 2).

In in vivo experiments, MDL 72222 was tested against a submaximal dose of the ganglionic muscarinic receptor stimulant, McN-A-343, in the pithed rat (Giachetti et al. 1982). Intravenous administration of MDL 72222, 0.28 or 1 mg/kg (compare Figs. 4A and 5a), had only minimal effects on the increase in heart rate and the rise in blood pressure evoked by McN-A-343 (Fig. 7). Thus, MDL 72222 does not affect a depolarizing stimulus arising from activation of neuronal muscarinic receptors. Moreover, since the effects of McN-A-343 are manifested entirely through actuation of the sympathetic nervous system, the compound, at the doses used, had no appreciable adrenoceptor or adrenergic neurone blocking activity in vivo.

MDL 72222 was tested for local anaesthetic activity in the guinea-pig wheal test of Bülbring and Wajda (1945). MDL 72222 (ED_{50}, 2.3 mmol/l) proved slightly less potent than either lignocaine (ED_{50}, 1.7 mmol/l)) or (−)-cocaine (ED_{50}, 1.8 mmol/l) in this test.

Discussion

5-HT receptors are present on many types of neurone in the vertebrate and invertebrate nervous systems and mediate a wide range of excitatory and inhibitory responses (Aghajanian 1981; Gerschenfeld et al. 1981; Wallis 1981). In the mammalian peripheral nervous system, Wallis (1981) differentiates two types of 5-HT receptor mediating neuronal excitation: one is present on the cell bodies and terminal of certain postganglionic sympathetic neurones and on primary afferents of the nodose ganglion; a second, the

Table 1. Selectivity of action of MDL 72222

Agonist	Rabbit heart	Rat uterus	Rat aorta	Rat fundus	Guinea-pig ileum	Guinea-pig taenia caeci[a]
5-HT	0.82	25,440	46,000	> 50,880	2,230	
DMPP	840				3,740	
Oxytocin		> 50,880				
Acetylcholine				12,402	3,660	
Histamine					3,676	
Calcium						28,620

Figures are mean IC_{50} values in nmol/l ($n = 3-6$) obtained against repeated submaximal doses of agonists and cumulative addition of antagonist

[a] In tissues depolarized with 40 mmol/l potassium and stimulated with 100 µmol/l calcium

Table 2. Effects of MDL 72222 on the binding of various radioligands to brain tissue membranes

Receptor	Ligand	Concentration (nmol/l)	Species	Tissue	Non-specific binding in presence of		MDL 72222 (IC_{50} nmol/l)	Reference agent	IC_{50} (nmol/l)
					compound	concentration (µmol/l)			
α_1-Adrenoceptor	^{3}H-WB-4101	0.5	Rat	Cortex	Phentolamine	10	> 10,000	Ketanserin	37
α_2-Adrenoceptor	^{3}H-clonidine	1	Rat	Cortex	Phentolamine	10	> 10,000	Idazoxan	7
β-Adrenoceptor	^{3}H-DHA	1	Rat	Cortex	(−)-Propranolol	1	> 10,000	(−)-Propranolol	3
5-HT_1	^{3}H-5-HT	2	Rat	Frontal cortex	5-Methoxytryptamine	10	> 10,000	5-HT	6
5-HT_2	^{3}H-spiperone	1	Rat	Frontal cortex	Mianserin	1	> 10,000	Ketanserin	9
Benzodiazepine	^{3}H-diazepam	1	Rat	Cortex	Clonazepam	0.1	> 10,000	Diazepam	8
Histamine H_1	^{3}H-pyrilamine	2	Guinea-pig	Cerebellum	Mepyramine	0.3	3,800	Mepyramine	3

IC_{50} values were derived from at least two determinations each involving 3 to 5 concentrations of each agent evaluated in triplicate
^{3}H-DHA = ^{3}H-dihydroalprenolol

classical M-receptor of Gaddum and Picarelli (1957), is present on the cholinergic elements of the myenteric plexus of the guinea-pig ileum. The present study establishes MDL 72222 as a potent and highly selective antagonist of the first of these receptor types.

The organ used to quantify actions and interactions with the 5-HT receptor of the postganglionic sympathetic neurone is the isolated heart of the rabbit. In this tissue, 5-HT stimulates transmitter release from the terminal sympathetic fibres (Jacob and Poite-Bevière 1960; Fozard and Mwaluko 1976; Göthert and Dührsen 1979) by activation of specific receptor sites (Fozard and Mobarok Ali 1978b; Fozard 1979, 1983b; Fozard et al. 1979). From Fig. 1 it is clear that MDL 72222 is a powerful antagonist of the indirect sympathomimetic response to 5-HT of the rabbit heart. Inhibition cannot involve the intermediary β-adrenoceptor, since the indirect sympathomimetic responses to the nicotinic agonist, DMPP, were unaffected by concentrations of MDL 72222 many times those effective against 5-HT (Fig. 1). Moreover, MDL 72222 does not displace ^{3}H-dihydroalprenolol from its recognition site in membranes from rat brain cortex (Table 2). Blockade, therefore, must be presynaptic. Direct evidence for this appears in Fig. 2 where the initial overflow of radioactivity from the heart following injection of 5-HT, which corresponds qualitatively and temporally with the chronotropic response (Fozard and Mwaluko 1976; Fozard 1979), was suppressed selectively by perfusion with MDL 72222, 5 nmol/l. The logical interpretation of these findings is that MDL 72222 is a selective antagonist at the excitatory 5-HT receptor present on rabbit cardiac sympathetic nerves.

The Bezold-Jarisch effect of 5-HT in the anaesthetized rat can be used to quantify the response to activation of excitatory 5-HT receptors on afferent sensory fibres (Fozard 1982; Fozard and Host 1982). The abrupt, transient cardiac slowing seen following bolus doses of 5-HT given intravenously (Salmoiraghi et al. 1956; see Fig. 3) results from reflex stimulation of the vagus following activation of afferent fibre terminals located mainly in the right ventricle (Krayer 1961; Paintal 1973). There is no doubt that MDL 72222 is a potent inhibitor of the Bezold-Jarisch effect of 5-HT, given either intravenously (Figs. 3, 4A and 5A) or into the duodenum (Fig. 5B). Moreover, since responses to electrical stimulation of the efferent vagus were unaffected by high doses of MDL 72222 (Fig. 4B) and the compound has only weak antimuscarinic activity in vitro (Fig. 6;

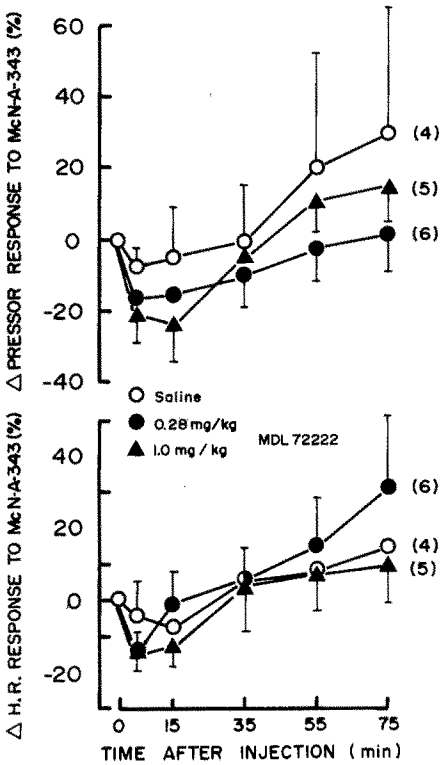

Fig. 7. Effects of MDL 72222 on the cardiovascular response to a submaximal dose of McN-A-343 (25–50 μg/kg) injected intravenously at 10–20 min intervals in the pithed, acutely adrenalectomized rat. MDL 72222 was injected intravenously at time 0 at the doses indicated. *Points* represent the mean values with standard errors of the number of individual observations shown in parentheses. The mean initial increases in blood pressure and heart rate evoked by McN-A-343 were 31 ± 3 mm Hg and 76 ± 11 beats/min for the group injected with saline and 42 ± 3 mm Hg and 76 ± 11 beats/min and 57 ± 15 mm Hg and 99 ± 12 beats/min for the groups receiving MDL 72222 0.28 mg/kg and 1 mg/kg, respectively

Table 1) and in vivo (Fig. 7)), the effects of MDL 72222 can be localized to the afferent pathway of the reflex. The data are consistent with blockade by MDL 72222 of the excitatory 5-HT receptors present on the vagal afferent fibres.

The response of the guinea-pig ileum to 5-HT in the presence of methysergide is exclusively indirect, the result of acetylcholine release from the postganglionic neurones of the intramural plexus (Day and Vane 1963; Fozard and Mobarok Ali 1978b). The response reflects activation of the neuronal receptors defined originally as M by Gaddum and Picarelli (1957). In direct contrast to the data obtained on the rabbit heart or in the anaesthetized rat, MDL 72222 proved neither potent nor selective as an antagonist of the neuronally mediated responses to 5-HT on the guinea-pig ileum (Fig. 6; Table 1). Inhibition was seen only at high concentrations, was non-surmountable and could not be ascribed to a presynaptic mechanism since responses to acetylcholine and histamine were suppressed at broadly similar concentrations (Fig. 6; Table 1). Thus, no evidence for an interaction of MDL 72222 with 5-HT at the neuronal receptors of the cholinergic nerves of the guinea-pig ileum has been obtained.

The lack of affinity of MDL 72222 for the excitatory neuronal receptors of the ileum contrasts with its potency as an antagonist of 5-HT-evoked transmitter release from the heart and is consistent with different receptor types mediating the two responses. The data provide direct support for the similar earlier conclusion based on the selectivity of action of 5-HT agonists (Fozard and Mobarok Ali 1978b) and the differential blocking activity of nor-(−)-cocaine (Fozard 1983b; see also Wallis 1981). The results from the present study allow extension of this classification to include the exitatory receptors for 5-HT present on vagal afferent fibres. Clearly, in terms of their susceptibility to blockade by low doses of MDL 72222, these sites are similar to those present on rabbit cardiac sympathetic nerves and different from those on the cholinergic elements of the guinea-pig ileum.

The selectivity of action of MDL 72222 as an antagonist of certain neuronal stimulant responses to 5-HT is remarkable both with respect to responses mediated through other 5-HT receptors and in general. The lack of affinity for the 5-HT M receptor of the ileum has been mentioned above. Similarly, MDL 72222 has no significant antagonist activity at the smooth muscle 5-HT receptors of rat aorta, uterus or fundus (Table 1), nor does the compound displace radioligand binding to the 5-HT_1 or 5-HT_2 recognition sites in rat brain membranes (Table 2). Significant affinity for the inhibitory autoreceptor on brain 5-HT neurones can also be ruled out since in slices of rat frontal cortex whose 5-HT neuronal transmitter stores had been labelled by prior exposure to ^{3}H-5-HT, MDL 72222 at a concentration of 1 μmol/l neither affected transmitter release evoked by potassium nor interfered with depression of release evoked by 5-HT (D. N. Middlemiss, personal communication). With respect to receptors for other ligands, MDL 72222 has no meaningful blocking activity at muscarinic or nicotinic cholinoceptors, α- or β-adrenoceptors or histamine H_1-receptors (Tables 1 and 2).

The lack of effect of MDL 72222 on the indirect sympathomimetic response evoked by stimulation of ganglionic muscarinic receptors by McN-A-343 (Fig. 7) bears emphasis. It confirms the lack of muscarinic and adrenoceptor blocking activity in vivo. Moreover, since depolarization induced by activation of muscarine receptors is characteristically "weak" (Haefely 1972, 1974a; Wallis 1979) and in this respect similar to that seen with 5-HT (Haefely 1974b), the selectivity of action of MDL 72222 (compare Fig. 7 with Fig. 5A) suggests blockade at a specific receptor site and not suppression of a post-receptorial event coupling the depolarizing stimulus to transmitter release. Consistent with this conclusion is the fact that although MDL 72222 has local anaesthetic activity and is only marginally less active than lignocaine or (−)-cocaine in this respect, this is seen only at high (mmol/l) concentrations. Moreover, classical local anaesthetics, such as lignocaine or tetracaine, manifest only weak non-surmountable blockade of depolarization-induced transmitter release with, on the heart, responses to DMPP being consistently more sensitive to inhibition than those to 5-HT (Fozard et al. 1979).

Finally, in the context of selectivity, a comment on the residual ^{3}H-overflow seen in the rabbit heart after bolus injections of 5-HT in the presence of MDL 72222 (Fig. 2) is justified. The response is similar to that of tyramine in being slow to develop and subside, and identical to that seen after injection of 5-HT to hearts perfused with low Ca^{2+} Tyrode's-solution (Fozard and Mwaluko 1976) or receptor-desensitizing concentrations of 5-HT (Fozard 1979). The observa-

tion is thus consistent with MDL 72222 unmasking the "tyramine-like" transmitter release known to occur following high doses of 5-HT (see Fozard 1979) by selective blockade of the 5-HT neuronal receptor release mechanism.

There remains one further observation deserving of particular comment and that is the question of the nature of the antagonism of 5-HT by MDL 72222. Only on the heart is it reasonable to attempt to draw conclusions in this context and even here interpretation is difficult due to the conditions necessary to achieve consistent responses to 5-HT being less than optimal for the study of agonist/antagonist interactions (for detailed discussion, see Fozard 1983b). Nevertheless, with the lowest concentrations of MDL 72222 used, the log dose-response curve to 5-HT is shifted to the right and there is no depression of the maximum response (Fig. 1), observations typical of an interaction between a full agonist and a competitive agonist. On the other hand, the effects of the higher concentrations of MDL 72222 were clearly non-surmountable (Fig. 1). It would, however, be premature to conclude from this that blockade with the higher doses of MDL 72222 was non-competitive. Thus, the log dose-response curve to 5-HT on the rabbit heart is characteristically "bell-shaped" (Fozard and Mwaluko 1976), presumably reflecting the reversible desensitization by 5-HT of the receptors mediating the stimulant effect (Fozard and Mwaluko 1976; Fozard and Mobarok Ali 1978b; Fozard 1979; Göthert and Dührsen 1979). Under these circumstances, depression of the slope and maximum of the 5-HT log dose-response curve by the higher concentrations of MDL 72222 might well reflect the combined effect of 5-HT autoinhibition plus the antagonist effects of MDL 72222.

In conclusion, MDL 72222 is a potent and remarkably selective antagonist of responses mediated through the excitatory 5-HT receptors present on the terminal sympathetic neurones of the rabbit heart and on the neurones subserving the afferent limb of the Bezold-Jarisch reflex. The compound should provide a powerful tool to distinguish responses mediated through such sites. In this context, the classical M receptor of the guinea-pig ileum (Gaddum and Picarelli 1957) can readily be differentiated since MDL 72222 is neither potent nor selective as an antagonist of 5-HT at this site.

Acknowledgements. I thank Dr. M. Gittos for synthesizing MDL 72222, Marie-Françoise Beya, Martine Host, Jacqueline Kintz and Marie-Louise Part for expert technical assistance, Dr. P. Mallorga and Dr. D. N. Middlemiss for the radioligand binding data, Dr. M. Spedding for the evaluation of MDL 72222 on the taenia caeci and Claudine Kratz for manuscript preparation.

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Received October 27, 1983/Accepted December 12, 1983