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Hypotensive and α-adrenergic activities of 2-(2,3,6-trichlorophenylimino) and 2-(2,3-dichloro-6-methylphenylimino) imidazolidine, two potent derivatives of clonidine

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1 α-Adrenergic activities (hypotension, bradycardia, sedation) and affinities of clonidine and two newly substituted derivatives, 2-(2,3,6-trichlorophenylimino)imidazolidine (I) and 2-(2,3-dichloro-6-methylphenylimino)imidazolidine (II), were determined in various *in vivo* and *in vitro* models.

2 In anaesthetized normotensive rats, the intravenous −log doses (mol/kg) of clonidine, compound I and II required to decrease mean arterial pressure by 20% were 7.96, 8.39 and 7.79, respectively, and to reduce heart rate by 20% were 7.85, 8.23 and 7.91, respectively. Comparable potencies were obtained in vagotomized rats. Following vertebral arterial infusion of clonidine, compound I and II into anaesthetized cats, the −log doses for 20% diminution in mean arterial pressure were 8.72, 9.17 and 7.92, respectively.

3 In pithed normotensive rats, the intravenous −log doses (mol/kg) of clonidine, compound I and II required to elevate diastolic pressure by 50 mmHg were 7.49 , 7.80 and 8.04, respectively, whereas 50% of the maximal inhibition of electrical stimulation-induced tachycardia was obtained at −log doses of 8.20, 8.25 and 8.34, respectively.

4 In mice, the intraperitoneal −log doses (mol/kg) needed for a prolongation of the hexobarbitone-induced loss of the righting reflex by 100% were 6.51, 6.55 and 6.60 for clonidine, compound I and II, respectively.

5 Compounds I and II displayed a higher affinity for α_1- and α_2-adrenoceptors than clonidine, identified in rat cerebral membranes by [^{3}H]-prazosin and [^{3}H]-clonidine.

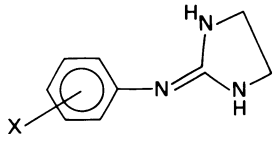
6 The results show that 2,3,6-trisubstituted derivatives of clonidine are potent α-adrenoceptor stimulants. This new class of congeners may have potential for pronounced hypotensive activity following systemic application. It is not likely that among members of this series, hypotensive potency can be separated from sedative activity.

Introduction

The hypothesis that the hypotensive activity of clonidine, 2-(2,6-di-chlorophenylimino)imidazolidine, is initiated within the central nervous system by stimulation of α_2-adrenoceptors at pontomedullary sites has gained widespread acceptance (Van Zwieten, 1975; Schmitt, 1977; Kobinger, 1978; Van Zwieten & Timmermans, 1979).

Since the discovery of the pronounced hypotensive properties of clonidine (Hoefke & Kobinger, 1966), efforts have been made to optimize the substitution in the phenyl ring in order to study the relationship between molecular structure and hypotensive activity as well as to obtain more potent or therapeutically more useful derivatives (Laverty, 1969; Walland & Hoefke, 1974; Stähle, 1974; Hoefke, Kobinger & Walland, 1975; Hoefke, 1976; Rouot, Leclerc, Wermuth, Miesch & Schwartz, 1977; Timmermans & Van Zwieten 1977a,b,c). However, among the hundreds of clonidine-like compounds that have been synthesized, only a few have been found to have blood pressure lowering activity comparable with that of clonidine itself (for reviews see Timmermans, Hoefke, Stähle & Van Zwieten, 1980; Stähle, 1982).

Apart from the demands that are made upon lipophilicity which is an absolute requirement for high hypotensive activity (Timmermans, Brands &



I X = 2,3,6-tri-Cl

II X = 2,3,-di-Cl,6-CH_3

Figure 1 Structural formulae of 2-(2,3,6-trichlorophenyl)imino (I) and 2-(2,3-dichloro-6-methylphenylimino)imidazolidine (II), two newly substituted clonidine-like derivatives used in the present study.

Van Zwieten, 1977; Timmermans *et al.*, 1977c), it is a general observation within the class of clonidine-like imidazolidines that the most active molecules are found among the 2,6- and 2,3-disubstituted congeners (Timmermans *et al.*, 1980; De Jonge, Slothorst-Grisdijk, Timmermans & Van Zwieten, 1981b). By combining 2,3- and 2,6-substitution within one and the same molecule, we have speculated upon the possibility of obtaining clonidine-like compounds with pronounced hypotensive activity. 2-(Phenylimino)-imidazolidines possessing such a 2,3,6-trisubstitution pattern have not yet been described. The present paper reports on the α-adrenergic and cardiovascular activities of two representatives of this newly substituted class of derivatives, viz. 2-(2,3,6-trichlorophenylimino) (I) and 2-(2,3-dichloro-6-methylphenylimino)imidazolidine (II) (Figure 1). The activities of compounds I and II were compared with those of clonidine. Some data have been communicated in a preliminary account (Timmermans, De Jonge, Van Zwieten, De Boer & Speckamp, 1982).

Methods

Effects on mean arterial pressure and heart rate of anaesthetized normotensive cats and rats

Cats of either sex, weighing 2–4 kg, were anaesthetized with α-glucochloralose (60 mg/kg, i.p.) and placed on thermostat-equipped tables to maintain body temperature at approximately 37°C. The animals were artificially ventilated via a tracheal cannula and subjected to left-sided thoracotomy. After cannulation of the left axillary artery, a catheter was pushed forward until its tip lay just distal from the ostium of the left vertebral artery. All other side-branches of the subclavian artery were ligated. The details of the method have been described elsewhere (Van Zwieten, 1975). A femoral artery and vein were catheterized for blood pressure measurements and administration of drugs, respectively. Heparin was injected i.v. (about 1000 iu/kg). After 30 min of equilibration, single doses of the compounds were infused either via the left vertebral artery (v.a.) or via the femoral vein (i.v.) in a volume of 140 μl over a period of 1 min. Log dose-response curves were constructed for the maximal decrease in mean arterial pressure (% of initial value) and −log dose for 20% decrease, pC_{20} BP was calculated.

Male Wistar normotensive rats weighing between 200 and 250 g were anaesthetized with pentobarbitone (75 mg/kg, i.p.) and ventilated via a tracheal cannula. Catheters were inserted into an external jugular vein and a common carotid artery for drug application and measurements of arterial pressure and heart rate which were recorded continuously. Following a period of 15 min of stabilization, single doses of the compounds were injected in a volume of 0.1 ml/100 g of body weight and the maximal fall in mean arterial pressure measured. In order to quantify the bradycardic activity, the test substances were injected cumulatively at 5 min intervals. Maximal decreases in mean arterial pressure or heart rate were plotted against log dose and −log dose for 20% reduction in mean arterial pressure, pC_{20}(BP), or cardiac frequency, pC_{20}(HR), were calculated.

In a separate series of experiments, anaesthetized rats (see above) were vagotomized bilaterally in the cervical region and treated with atropine (1 mg/kg, s.c.). The decrease in heart rate was quantified following intravenous administration of the compounds in a cumulative manner. Bradycardic activity in vagotomized rats was expressed by means of a pD_2 value, calculated from the log dose-response curves.

Effects on diastolic pressure and stimulation-induced tachycardia in pithed normotensive rats

Male normotensive rats were lightly anaesthetized with diethyl ether, their trachea cannulated, pithed via the orbit (Gillespie, McLaren & Pollock, 1970) and placed on heated tables. Immediately thereafter, artificial respiration with room air was started. The pithing rod was insulated throughout its length apart from a 1 cm section about 6 cm from the tip. Arterial pressure was recorded via a cannulated common carotid artery and heart rate derived from the pressure pulse. A jugular vein was used for drug injections via which heparin (about 1000 iu/kg) was administered.

In non-stimulated preparations, test substances were injected as single doses in a volume of 0.5 ml/kg about 20 min after completion of the surgical intervention. Each pithed rat received 3–4 doses in random order. The maximal increase in diastolic pressure was measured and plotted against log dose. Hypertensive potency was expressed as −log dose for an increase in diastolic pressure of 50 mmHg, pC_{50}.

In order to prepare the pithed rats for continuous electrical stimulation of the preganglionic sympathetic cardioaccelerator nerves, an indifferent electrode was placed dorsally in the neck, atropine and tubocurarine (1 mg/kg of each, i.v.) were given and both vagal nerves were cut. Following a period of stabilization (about 20 min), stimulation of the spinal cord (C7–Th1) was achieved by means of rectangular pulses (0.2 Hz, 2 ms, 50 V) between the non-insulated segment and the indifferent electrode. The position of the pithing rod was adjusted until a sustained increase in heart rate of 70–80 beats/min was obtained with virtually no change in mean arterial pressure. The compounds were injected cumulatively in a volume of 0.5 ml/kg at 5 min intervals. The decrease in cardiac frequency was quantified and expressed as percentage inhibition of stimulation-induced tachycardia. Log dose-response curves were made and activity was calculated as pD_2 values.

Effect on hexobarbitone-induced loss of righting reflex in mice

Male albino mice (20–30 g) were injected with hexobarbitone-sodium (75 mg/kg, i.p.) 15 min after intraperitoneal treatment with saline or the compounds to be tested. The loss of the righting reflex was measured in min ($n=10$ per group). Sleeping animals were kept on heated tables to maintain body temperature at about 38°C. All substances were administered in a volume of 0.1 ml/10 g of body weight. Sedative activity was calculated as −log dose prolonging the hexobarbitone sleeping time by 100% pC_{100}.

Effect on specific [^{3}H]-clonidine and [^{3}H]-prazosin binding in rat isolated brain membranes

A crude membrane suspension of rat brain was prepared according to reported standard procedures (Greenberg, U'Prichard & Snyder, 1977; U'Prichard, Greenberg & Snyder, 1977). Protein concentration, as determined by the method of Lowry, Rosebrough, Farr & Randall (1951), was 4 mg/ml for the [^{3}H]-clonidine and 1 mg/ml for the [^{3}H]-prazosin binding assays. Aliquots of 0.5 ml were mixed with [^{3}H]-clonidine (sp. act. 26.7 Ci/mmol; 0.4n M) or ([^{3}H]-prazosin (sp. act. 33 Ci/mmol; 0,2n M) and various concentrations of the compounds in 50 m M Tris/HCl buffer (pH=7.7 at 25°C) to a total volume of 1 ml. Incubations were run in duplicate at 25°C for 30–45 min and terminated by rapid vacuum filtration through Whatman GF/B filters. Filters were washed with three successive 5 ml portions of ice-cold buffer, solubilized in 10 ml of Instagel and counted. The specific binding of [^{3}H]-clonidine and [^{3}H]-prazosin was defined as the excess over blanks containing 10 μM (−)-noradrenaline and 2 μM phentolamine, respectively. The concentration of the compounds inhibiting 50% of the specific binding of each radioligand was calculated by log probit analysis of the binding data.

Drugs and chemicals

2-(2,3,6-Trichlorophenylimino) (I) and 2-(2,3-dichloro-6-methylphenylimino) imidazolidine hydrochloride (II) were prepared by Dr J.J.J. de Boer (Department of Organic Chemistry, University of Amsterdam); atropine sulphate and (−)-noradrenaline hydrochloride (Sigma); α-glucochloralose (Merck); heparin (NOVO); hexobarbitone-sodium (Bayer); Instagel (Packard-Becker); pentobarbitone (Nembutal, Abbott); phentolamine hydrochloride (Ciba-Geigy); clonidine and [^{3}H]-clonidine hydrochloride (Boehringer Ingelheim); [^{3}H]-prazosin hydrochloride (Pfizer); tubocurarine (Burroughs Wellcome). α-Glucochloralose was taken up in hot distilled water. All other drugs were dissolved in saline. When appropriate, doses mentioned in the text refer to the form indicated above.

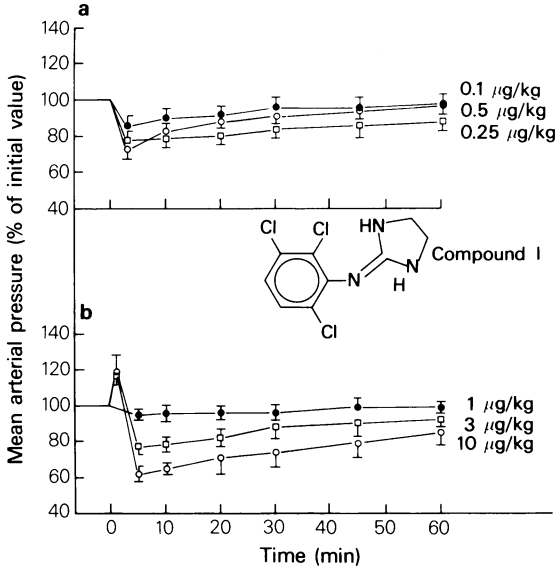


Figure 2 Effect of 2-(2,3,6-trichlorophenylimino) imidazolidine (I) on mean arterial pressure (% of initial value) of chloralose anaesthetized cats. The drug was infused via the left vertebral artery (a) or intravenously (b) starting at t=0 min over a period of 1 min. Symbols represent mean values (n=5); s.e.means shown by vertical lines. Initial value of mean arterial pressure was 125 ± 5 mmHg ($n=30$).

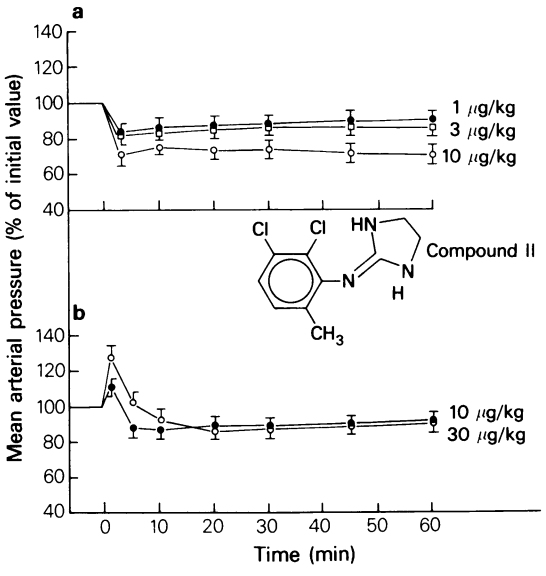


Figure 3 Effect of 2-(2,3-dichloro-6-methylphenylimino)imidazolidine (II) on mean arterial pressure (% of initial value) of chloralose-anaesthetized cats. The drug was infused via the left vertebral artery (a) or intravenously (b) starting at t=0 min over a period of 1 min. Symbols represent mean values ($n=5$); s.e.means shown by vertical lines. Initial value of mean arterial pressure was 134 ± 6 mmHg ($n=30$).

Results

Hypotension and bradycardia in anaesthetized normotensive cats and rats

Following infusion of clonidine (0.1–2 μg/kg) via the left vertebral artery of anaesthetized cats, the well-known, pronounced depressor effects of this drug (Sattler & Van Zwieten, 1967; Timmermans & Van Zwieten, 1977b) were confirmed in the present study. The time-course of the hypotensive effects of the derivatives I and II after v.a. or i.v. infusion into this animal species are depicted in Figures 2 and 3. Table 1 lists the central hypotensive potencies, pC_{20} (BP), of the compounds. The two substances I and II were very effective in lowering mean arterial pressure upon v.a. infusion. In this respect, the 2,3,6-trichloro-substituted compound I was found to be 3 times more potent than clonidine. As indicated by the data enumerated in Table 1, the hypotensive activities of all three drugs was much less after i.v. administration, illustrating the central nervous origin of the depressor response.

Clonidine and its two congeners I and II produced qualitatively comparable effects on mean arterial pressure after i.v. injection into anaesthetized normotensive rats. There was an initial transient rise in pressure followed by a prolonged decrease. Figure 4 shows the log dose-response curves with respect to the maximal reduction in mean arterial pressure. The hypotensive potencies, expressed as pC_{20}(BP), calcu-

Table 1 α-Adrenergic potencies (hypotension, bradycardia, sedation) and affinities of clonidine and its two derivatives 2-(2,3,6-trichlorophenylimino) (I) and 2-(2,3-dichloro-6-methylphenylimino)imidazolidine (II) determined in various *in vivo* and *in vitro* models

	Blood pressure		*Heart rate* (rat)		*Pithed rat*		*Sedation*	*Affinity* IC50	
	rat	cat	intact	vagotomized			(mice)		
Compound	pC_{20}[a]	pC_{20}[b]	pC_{20}[c]	pD_2[d]	pC_{50}[e]	pD_2[f]	pC_{100}[g]	(α_1)[h]	(α_2)[i]
Clonidine	7.96	8.72	7.85	8.40	7.49	8.20	6.51	488	3.20
I	8.39	9.17	8.23	8.68	7.80	8.25	6.55	290	2.72
II	7.79	7.92	7.91	8.50	8.04	8.34	6.60	138	2.32

[a] –log dose (mol/kg) to decrease mean arterial pressure by 20% following i.v. administration to anaesthetized normotensive rats.

[b] –log dose (mol/kg) to decrease mean arterial pressure by 20% following infusion into the vertebral artery of anaesthetized cats.

[c] –log dose (mol/kg) to decrease heart rate by 20% following cumulative i.v. administration to anaesthetized normotensive rats.

[d] –log dose (mol/kg) for 50% of the maximal decrease in heart rate following cumulative i.v. administration to vagotomized anaesthetized normotensive rats.

[e] –log dose (mol/kg) to increase diastolic pressure by 50 mmHg following i.v. administration to pithed normotensive rats.

[f] –log dose (mol/kg) for 50% of the maximal inhibition of electrical stimulation-induced tachycardia following cumulative i.v. administration to pithed normotensive rats.

[g] –log dose (mol/kg) prolonging the hexobarbitone (75 mg/kg, i.p.) induced loss of the righting reflex in mice by 100% following i.p. administration.

[h] Concentration (nM) inhibiting the specific binding of [^{3}H]-prazosin (0.2 nM) to rat brain membranes by 50%

[i] Concentration (nM) inhibiting the specific binding of [^{3}H]-clonidine (0.4 nM) to rat brain membranes by 50%.

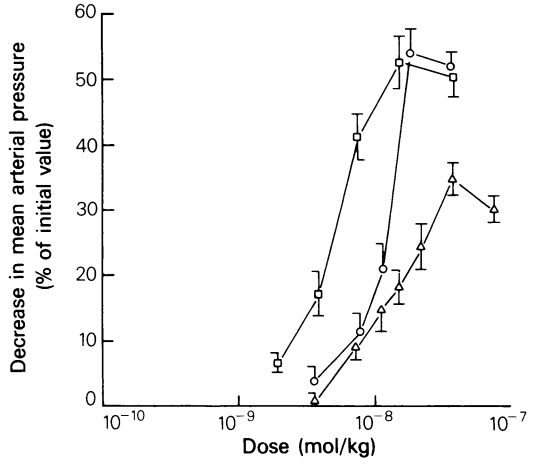


Figure 4 Log dose-response characteristics of the maximal decrease in mean arterial pressure (% of initial value) following i.v. administration of clonidine (2,6-di-Cl) (○) and its two derivatives I (2,3,6-tri-Cl) (□) and II (2,3-di-Cl,6-CH_3) (△) to pentobarbitone-anaesthetized normotensive rats. Data are mean values ($n=5$); s.e.means shown by vertical lines. The initial preinjection value of the mean arterial pressure of the animals was 121 ± 4 mmHg ($n=85$).

lated from these curves are listed in Table 1. The 2,3,6-trichloro analogue I was about 3 times more effective than clonidine and compound no II was slightly less active than clonidine. The maximal hypotensive effects of clonidine and compound I were virtually the same (about 53% decrease), whereas that of compound II amounted to approximately 35%.

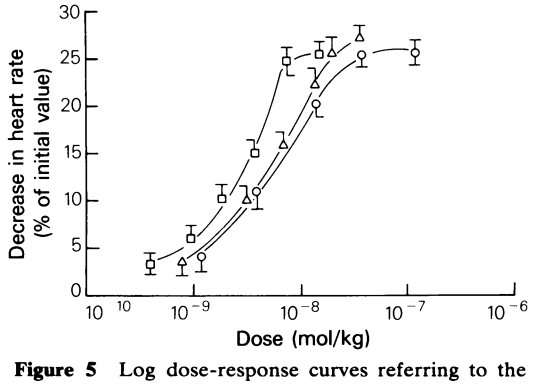


Figure 5 Log dose-response curves referring to the maximal decrease in cardiac frequency (% of initial value) after cumulative i.v. injections of clonidine (2,6-di-Cl) (○) and compounds I (2,3,6-tri-Cl) (□) and II (2,3-di-Cl,6-CH_3) (△) into pentobarbitone-anaesthetized normotensive rats (means and s.e.means, $n=5$-6). The initial mean heart rate was 402 ± 6 beats/min ($n=17$).

The fall in arterial pressure of the anaesthetized normotensive rats caused by the imidazolidines was accompanied by a dose-dependent decrease in heart rate. This bradycardic effect, quantified by cumulative administration, has been depicted in Figure 5 by means of log dose-response curves. Table 1 lists the pC_{20} (HR) values. Compound I was again somewhat more effective than clonidine and compound II.

In vagotomized, atropine-treated, pentobarbitone-anaesthetized normotensive rats, the maximal reduction in cardiac frequency was 18% of the initial value for all three substances after i.v. application. The log dose-bradycardic response curves are given in Figure 6. The corresponding pD_2 values are shown in Table 1. As with the findings described above, the 2,3,6-trichlorosubstituted molecule showed the highest potency.

Pressor and presynaptic effects in pithed normotensive rats

Following i.v. injections into pithed normotensive rats, clonidine and its two congeneric drugs increased diastolic pressure in a dose-dependent fashion. The complete log dose-response characteristics referring to the maximal pressor effects are depicted in Figure 7. The calculated pC_{50} values are tabulated in Table 1. For all three imidazolidines the maximal response amounted to an increase in diastolic pressure of approximately 100 mmHg. In this animal preparation, both tri-substituted compounds had clearly

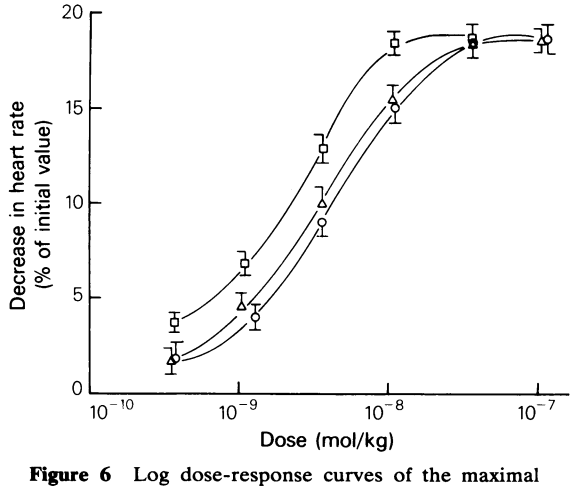


Figure 6 Log dose-response curves of the maximal decrease in cardiac frequency (% of initial value) brought about by clonidine (2,6-di-Cl) (○), derivative no I (2,3,6-tri-Cl) (□) and no II (2,3-di-Cl, 6-CH_3) (△) after cumulative i.v. application to vagotomized, atropine-treated, normotensive rats anaesthetized with pentobarbitone. Points are mean values ($n=6$); vertical lines show s.e.mean. The initial mean heart rate amounted to 426 ± 3 beats/min ($n=18$).

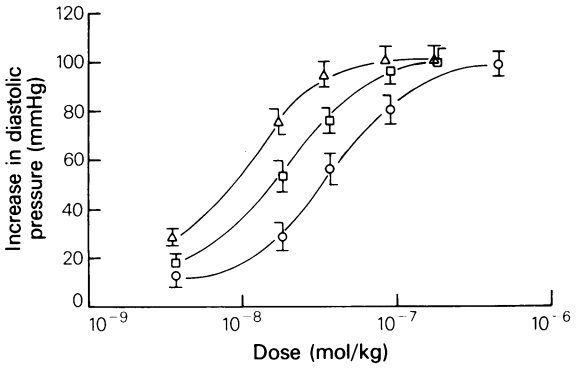


Figure 7 Log dose-response curves with respect to the maximal increase in diastolic pressure caused by i.v. administration of clonidine (2,6-di-Cl) (○) compound no I (2,3,6-tri-Cl) (□) and no II (2,3-di-Cl,6-CH_3) (△) to pithed normotensive rats. Symbols are given as mean values ($n=5$); s.e.mean shown by vertical lines. The mean preinjection diastolic pressure of the pithed animals was 41.8 ± 1.2 ($n=25$).

more pronounced hypertensive activities than clonidine.

Continuous electrical stimulation of the cardiac sympathetic nerves of pithed normotensive rats provoked an increase in heart rate of 83 ± 5 beats/min (mean ± s.e.mean, $n=17$). Upon cumulative i.v. application of the compounds, the stimulation-induced tachycardia was dose-dependently inhibited (Figure 8). Presynaptic activity, expressed as pD_2, is shown in Table 1. Compound II displayed a comparable intrinsic activity to clonidine (0.80). Compound I had a higher intrinsic activity (0.95).

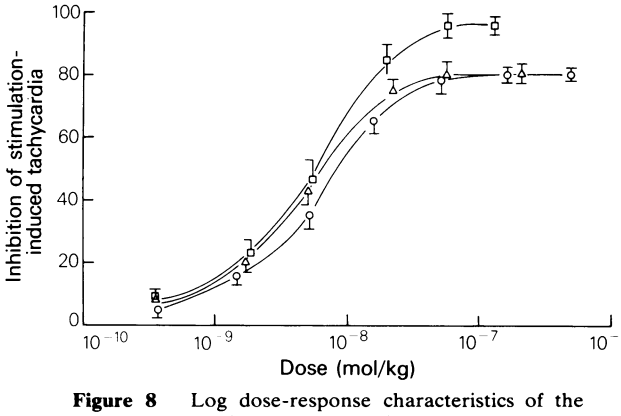


Figure 8 Log dose-response characteristics of the inhibitory effect (%) of clonidine (2,6-di-Cl) (○), structure no I (2,3,6-tri-Cl) (□) and no II (2,3-di-Cl, 6-CH_3) (△) on the tachycardia elicited by electrical stimulation (0.2 Hz, 2 ms, 50 V) of the spinal sympathetic efferents to the heart of pithed normotensive rats. Data are presented as mean values ($n=5$–6); s.e.mean shown by vertical lines. The compounds were injected i.v. in a cumulative manner.

Potentiation of hexobarbitone-induced sleeping time in mice

Control mice slept 12.4 ± 1.2 min ($n=30$) following an i.p. injection of hexobarbitone (75 mg/kg). All three drugs caused a dose-dependent prolongation of the sleeping time. Log dose-response curves were constructed for the increase in sleeping time (% of control) and the −log dose (mol/kg) prolonging the duration of the loss of the righting reflex by 100%, pC_{100}, was determined graphically. The data in Table 1 show that in this test, clonidine and compounds I and II had similar potencies as sedatives.

Inhibition of the specific binding of [^{3}H]-clonidine and [^{3}H]-prazosin to rat brain membranes

Specifically bound [^{3}H]-clonidine as well as [^{3}H]-prazosin was inhibited by the drugs according to sigmoid displacement curves (Figure 9). The concentration of the compounds required to reduce the specific binding of both radioligands by 50% are listed in Table 1. Clonidine and its two congeners showed highest binding affinity for the specific sites labelled with [^{3}H]-clonidine. Compounds I and II proved slightly more effective in competing for the [^{3}H]-clonidine binding sites than nonradioactive clonidine itself. The affinities of the three drugs for the specific binding sites occupied by [^{3}H]-prazosin were about 100 times lower. In this respect, compound II was twice as active as compound I and about 4 times more effective than clonidine.

Discussion

There is a vast body of experimental evidence in support of the view that the hypotension elicited within the central nervous system by clonidine and related drugs is due to stimulation of α-adrenoceptors of the α_2-type (Berthelsen & Pettinger, 1977; Timmermans Schoop, Kwa & Van Zwieten, 1981; Kobinger & Pichler, 1982). In the qualitative and quantitative studies aiming at correlating the central hypotensive activity (= α_2-adrenoceptor stimulating potency) with the molecular structure of clonidine-like imidazolidines, distinct features for an optimal interaction between 2-(phenylimino)imidazolidine and central α_2-adrenoceptor have emerged (Stähle, 1974; Hoefke, 1976; Timmermans & Van Zwieten, 1977c; Timmermans *et al.*, 1980; Stähle, 1982). Apart from changes in the 5-membered imidazolidine portion and the ring junction of these molecules, variations in the positions and character of the substituents attached to the phenyl nucleus greatly influence central hypotensive activity (for review see Timmermans *et al.*, 1980). 2-

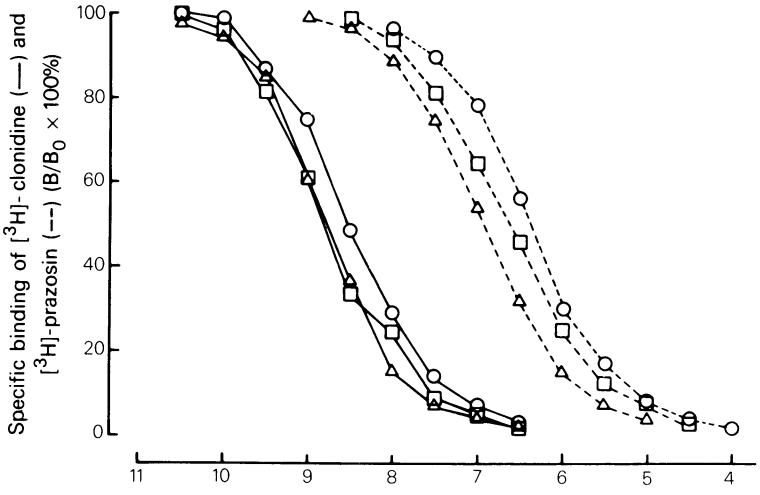


Figure 9 Displacement of [^{3}H]-clonidine (0.4 nM and [^{3}H]-prazosin (0.2 nM) from their specific binding sites in rat isolated brain membranes by increasing concentrations (M) of unlabelled clonidine (○), compound I (2,3,6-tri-Cl) (□) and II (2,3-di-Cl, 6-CH_3) (△). Means of four determinations performed in duplicate. B, fraction of radioligand specifically bound in the presence of competing displacer; B_0, in the absence of competing displacer.

(Phenylimino)imidazolidines possessing high hypotensive activity are preferably 2,6- or 2,3-disubstituted with substituents such as Cl, Br or CH_3. The 4-position only allows for small substituents, like OH and NH_2 (Timmermans & Van Zwieten, 1977c; Leclerc, Rouot, Schwartz, Velly & Wermuth, 1980). It should be stressed that in all these considerations the ability of the molecules to penetrate into the central nervous system is a prerequisite for pronounced hypotensive activity. Thus, lipophilicity of the drugs greatly determines central (hypotensive) activity following systemic application. For instance, *para*-aminoclonidine is a very potent α-adrenoceptor stimulant, in fact more active than clonidine itself, but behaves as a rather moderately effective hypotensive drug due to its unfavourable lipophilicity (Rouot & Snyder, 1979; Leclerc *et al.*, 1980; authors' unpublished observations). With increasing steric bulk at the 5-position hypotensive activity decreases (De Jonge *et al.*, 1981b). It is an interesting finding that when the steric dimensions at this 5-position grow too large, the derivative completely loses the ability to stimulate α_2-adrenoceptors and becomes a very selective α_1-adrenoceptor agonist (De Jonge, Van Meel, Timmermans & Van Zwieten, 1981c). Characteristics of the structure-activity relationship in α-adrenergic 2-(phenylimino)imidazolidines of the clonidine type have also been described for α-adrenoceptors at cholinergic nerve terminals in the guinea-pig ileum inhibiting acetylcholine release (Malta, Raper & Tawa, 1981), at postsynaptic sites in the rat anococcygeus muscle (Chapleo, Doxey, Myers, Roach & Smith, 1981), rat (Ruffolo, Waddell & Yaden, 1980) and guinea-pig aorta (Medgett & McCulloch, 1980), rabbit ear artery (Hieble & Pendleton, 1979) as well as at presynaptic locations in the rat vas deferens (Chapleo *et al.*, 1981) in guinea-pig atria (Medgett & McCulloch, 1980) in the rabbit ear artery (Hieble & Pendleton, 1979) and in the rat superior cervical ganglia (Medgett, 1982).

In view of the foregoing it is obvious that the possibilities for optimizing the substitution pattern in clonidine-like imidazolidines with the purpose of obtaining potent hypotensive drugs are limited to the 2-, 3- and 6-position. Molecules possessing substituents at all these three positions have not been described yet. In theory, 2,3,6-trisubstituted derivatives are very attractive, since highest hypotensive activity is found among the 2,3-and the 2,6-disubstituted analogues (see above). The two new structures I and II described in the present paper show that members of this class of imidazolidines are potentially highly active hypotensive agents when administered systemically, particularly the 2,3,6-trichlorosubstituted derivative which was found more potent than clonidine in causing cardiovascular depression in anaesthetized cats and rats. The higher activity of this particular derivative over clonidine can be explained at least partly by its increased lipophilicity due to the introduction of an additional Cl substituent at the 3-position.

In addition to the α_2-adrenoceptor-mediated actions elicited within the central nervous system, compounds I and II were also very effective stimulants of cardiac presynaptic α_2-adrenoceptors. It is intriguing that the introduction of the 3-chloro substituent in

clonidine increased intrinsic activity at the presynaptic α_2-adrenoceptor in the rat heart. Furthermore, the radioligand binding experiments using [^{3}H]-prazosin to label α_1-adrenoceptors (Greengrass & Bremner, 1979) and [^{3}H]-clonidine to identify α_2-adrenoceptors (Greenberg *et al.*, 1977; U'Prichard *et al.*, 1977) also detected a slight increase in affinity of the two derivatives over clonidine with respect to both classes of α-adrenoceptors.

Both drugs I and II were more effective vasoconstrictor agents than clonidine as indicated by their hypertensive activity in pithed normotensive rats. The occurrence of vasoactive α_1- as well as α_2-adrenoceptors at postsynaptic locations in vascular smooth muscle is now well documented (for reviews see Starke, 1981; Timmermans & Van Zwieten, 1981; McGrath, 1982). Concomitantly, both α_1- and α_2-adrenoceptor-stimulating activity of the derivatives I and II may be more pronounced than that of clonidine. In view of the comparable presynaptic cardiac α_2-adrenergic potencies of clonidine and compound II (see above) and the close similarity of pre- and postsynaptic α_2-adrenoceptors (De Jonge, Santing, Timmermans & Van Zwieten, 1981), it may be concluded that the gain in pressor potency of compound II over clonidine is due to an increased ability of the former to activate α_1-adrenoceptors.

Sedative effects of clonidine measured in various species have been postulated to result from an action on central α_2-adrenoceptors (Drew, Gower & Marriott, 1979; Timmermans *et al.*, 1981; Pichler & Kobinger, 1981) possibly at a presynaptic location (Strömbom, 1975; Zebrowska-Lupina, Przegalinski, Stoniec & Kleinrock, 1977). It is a general observation that within clonidine-like imidazolidines, sedative activity parallels hypotensive potency (Timmermans *et al.*, 1980). The present study shows that the sedative potential of the two derivatives I and II is also particularly great. This indicates that in the present series of compounds also a separation between the sedative and hypotensive potency is not to be expected.

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