Br. J. Pharmacol. (1988), **94**, 19–26

4-Hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH): a new potentiator of sympathomimetic amines on the rat anococcygeus muscle

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1 The potentiating effects of racemic 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH), cocaine, desipramine and nomifensine on the concentration-response curves of the rat anococcygeus muscle to noradrenaline (NA) and field stimulation were examined.

2 PI-OH and cocaine concentration-dependently potentiated the responses of anococcygeus muscle to NA and field stimulation, but the activity of PI-OH was stronger than that of cocaine on both responses.

3 At high concentrations the potentiating activities of desipramine and nomifensine were less, a fact that was explained by their postsynaptic inhibitory properties; the actions of nomifensine and desipramine as antagonists against NA were competitive and non-competitive, respectively.

4 It is concluded that PI-OH may be an ideal potentiator of the response to NA in adrenergically-innervated tissues because it has no side effects such as postsynaptic inhibition.

Introduction

Preparations of isolated anococcygeus muscle of rats appear to offer advantages over other sympathetically innervated isolated tissues in pharmacological research and have been used successfully in studies on the actions of drugs on α-adrenoceptors and on catecholamine release and uptake processes (Gillespie, 1972; Gibson & Gillespie, 1973; Nash *et al.*, 1974; Doggrell & Woodruff, 1977). Many tricyclic antidepressants have been reported to potentiate the response of the rat anococcygeus muscle to noradrenaline (NA), possibly due to inhibition of neuronal uptake. They usually have potentiating effects at low concentrations of 10^{-7} M or 10^{-6} M, but cause no potentiation at 10^{-5} M, presumably because at the high concentration their α-adrenoceptor blocking activity becomes significant (Doggrell & Woodruff, 1977; Kenakin & Beek, 1981; Leighton, 1982). We have found a new compound: racemic 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH) which has a potent potentiating effect on the response of rat anococcygeus muscle to NA without any inhibitory effect at higher concentrations. This compound is an analogue of the antidepressant, nomifensine (Hoffman, 1973), which has a hydroxy group at the 4-position and no amino group at the 8-position of the nomifensine structure (Figure 1).

The present paper describes the potentiating activity and potency of PI-OH on the NA-induced contractile response of the rat anococcygeus muscle in comparison with those of cocaine, desipramine and nomifensine.

Methods

Male Wistar rats (200–250 g) were stunned and killed by exsanguination. The two anococcygeus muscles from each animal were excised as described by Gillespie (1972). Each muscle was suspended in a 10 ml organ bath containing Tyrode solution (mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 10.0) instead of Krebs solution as used by Gillespie (1972). The bath was maintained at 37°C and bubbled with air. The initial

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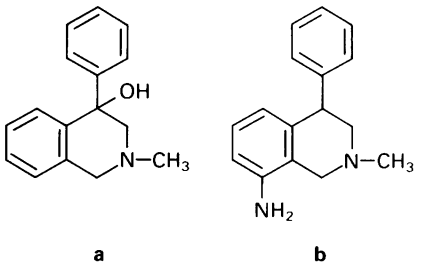

a b

Figure 1 Structural formulae of 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH) (a) and nomifensine (b).

resting tension was adjusted to 0.3–0.5 g, and the response to agonists was recorded with an isotonic transducer (Natume KN-259) and a pen recorder. When the intramural nerve fibres were to be stimulated, the muscle was drawn through a pair of platinum loop electrodes and stimulated by square pulses of 0.5 or 1 ms duration at 5 Hz and at supramaximum voltage from the stimulator (Nihon Kohden MSE-3R), in such a way that the degrees of twitch contraction were adjusted to about 25% of the maximum contraction to NA by a minor change of train duration or frequency (Hz).

Measurement of potentiating activity

Four compounds were tested: PI-OH, cocaine, desipramine and nomifensine. A cumulative method was used and potentiating activity was measured by recording the response curves for each agonist in the absence and presence of a test compound. After equilibrating the muscles for 2 h with repeated washing, the cumulative responses to an agonist were recorded 2 to 3 times at intervals of 30 min until the responses were the same in two successive recordings either in the absence or presence of the test compound. The exposure time of the muscles to the test compound was 30 min for each recording. Contractions were expressed as percentages of the maximum response to each agonist used. A regression line was calculated for each concentration-response curve and from each such line the pD_2 value (negative logarithm of the molar concentration of agonist producing 50% of the maximum response) was calculated (van Rossum, 1963). A test compound that had a potentiating effect on the response to an agonist caused a shift in the concentration-response curves at lower concentrations of the agonist. The ability of a drug to potentiate the action of an agonist is expressed as the concentration-ratio (fold), determined as the antilogarithm of the difference between the pD_2 values for the agonist obtained in the presence and absence of the test compound. When compounds had inhibitory effects at high concentrations but caused potentiation at low concentrations, their α-adrenolytic property and potency were examined more carefully.

Determination of postsynaptic antagonism

Postsynaptic antagonist activity was assessed by determining the pA_2 values against NA on the anococcygeus muscle in the presence of cocaine 10^{-5} M and propranolol 10^{-7} M. Concentration-ratios were determined from the difference between the concentration-response curves to NA in the absence and presence of antagonist, the latter being shifted to the right. Results are shown as Schild plots (Arunlakshana & Schild, 1959) and the pA_2 and slope of the regression were calculated. When the maximum response to NA was depressed in the presence of an antagonist, the pD'_2 value was estimated as indicating non-competitive antagonism (van Rossum, 1963).

Unless otherwise stated, all concentrations used are expressed as mol per litre and values given are means ± s.e. mean with the number of determinations in parentheses.

Materials

The synthesis of 4-hydroxy-2-methyl-4-phenyl- 1,2,3,4-tetrahydroxyisoquinoline (PI-OH) was described by Hinton & Mann (1959). The racemic PI-OH used in this work was synthesized by another route by Kihara *et al.* (1987). The free base of PI-OH was dissolved in 0.1 N HCl to be 10^{-3} M and the requisite dilutions were made with Tyrode solution.

The other drugs used in this investigation were (−)-noradrenaline bitartrate (Wako Pure Chem. Ind.), acetylcholine chloride (Daiichi), cocaine hydrochloride (Takeda), (±)-propranolol (Sigma), desipramine hydrochloride (Ciba-Geigy), nomifensine maleate (Hoechst, Japan), tetrodotoxin (Sankyo), prazosin hydrochloride (Mitsubishi Chemical Industry), and guanethidine sulphate (Tokyo Kasei).

Results

Effects of drugs on the log concentration-response curves of noradrenaline

Noradrenaline (NA) caused powerful contractions of rat anococcygeus muscle, as previously described (Gillespie, 1972). In this work, the pD_2 values for NA were found to be 6.27 to 6.38 and maximum contractions were produced at 10^{-5} M, as shown in Table 1 and Figure 2. The effects of four drugs: PI-OH, cocaine, desipramine and nomifensine, on the response to NA were tested. None of the drugs

Table 1 Potentiating activities of 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH), cocaine, desipramine and nomifensine on the noradrenaline (NA) concentration-response curves of rat anococcygeus muscle

Concentrations of test drugs	*PI-OH* pD_2 values	(fold)[a]	*Cocaine* pD_2 values	(fold)
(*n*)[b]	(9)		(6)	
Absence	6.30 ± 0.02		6.33 ± 0.05	
3×10^{-7} M	7.11 ± 0.04	(6.8)		
10^{-6} M	7.43 ± 0.06	(14.7)	7.09 ± 0.04**	(5.8)
3×10^{-6} M	7.58 ± 0.05	(20.1)	7.38 ± 0.06*	(11.7)
10^{-5} M			7.48 ± 0.08	(11.9)
Concentrations of test drugs	*Desipramine* pD_2 values	(fold)	*Nomifensine* pD_2 values	(fold)
(*n*)	(6)		(6)	
Absence	6.38 ± 0.02		6.27 ± 0.04	
3×10^{-7} M	7.26 ± 0.03*	(9.0)	7.20 ± 0.02	(8.7)
10^{-6} M	7.50 ± 0.05	(14.7)	7.31 ± 0.06*	(11.3)
3×10^{-6} M	7.25 ± 0.08**	(8.7)	7.20 ± 0.04**	(8.6)

[a] 'Fold' shows the concentration-ratio obtained from the antilogarithm of the difference between the pD_2 values for NA obtained in the absence and presence of the test drugs, respectively.
[b] *n* shows the number of experiments.
[c] Asterisks indicate the levels of significance (unpaired *t* test) when compared with the value for PI-OH at each concentration (* $P < 0.05$; ** $P < 0.01$).

caused contraction of the muscle in the concentration-ranges tested.

The effect of PI-OH (3×10^{-7}, 10^{-6} and 3×10^{-6} M) on the NA response of the anococcygeus muscle is shown in Figure 2a. In the absence of PI-OH the pD_2 value obtained from the log concentration-response curve of NA was 6.30 ± 0.02. After incubation with PI-OH, the NA curves were shifted to the left in parallel and concentration-dependently. Maximum sensitization to NA occurred after treatment with 3×10^{-6} M PI-OH and the pD_2 value of NA was 7.58 ± 0.05 (20.1 fold). A higher concentration of PI-OH (10^{-5} M) caused slight contraction of the muscle so its potentiating activities could not be tested at high concentrations. Cocaine also potentiated the NA response as shown in Figure 2b. However, the concentrations of cocaine tested, 10^{-6}, 3×10^{-6} and 10^{-5} M, were rather higher than those of PI-OH. The maximum sensitization to NA was induced by cocaine at 10^{-5} M and the pD_2 value was 7.48 ± 0.08 (11.9 fold) as shown in Table 1. These results show that the potentiating activity of cocaine is less than that of PI-OH for NA.

The effects of desipramine and nomifensine on the NA curve are shown in Figure 2c and d and their potentiating activities on the NA curve were as shown in Table 1. At a low concentration of 3×10^{-7} M, the potentiation produced by desipramine and nomifensine was greater than that of PI-OH. Both drugs caused their maximum potentiation at a concentration of 10^{-6} M. At a higher concentration of 3×10^{-6} M, these effects were less than those at 10^{-6} M.

Postsynaptic inhibitory properties of test drugs

Preparations pretreated with cocaine 10^{-5} M and propranolol 10^{-7} M were used to test postsynaptic inhibitory properties. After incubation with nomifensine at 3×10^{-6} M to 3×10^{-5} M, the concentration-response curves of NA were shifted to the right in parallel and concentration-dependently (Figure 3b). From these results the pA_2 of nomifensine for α-adrenoceptors was calculated to be 5.94 ± 0.08 (6) and the linear Schild regression had a slope of 0.98 ± 0.10 (6). Because this slope was not significantly different from unity, nomifensine was a true competitive antagonist against NA. PI-OH up to 10^{-5} M did not have any inhibitory activity on the NA curve in the presence of cocaine and propranolol (Figure 3a).

Desipramine at a low concentration of 3×10^{-6} M produced only a slight parallel shift to the right of the NA curve, but at higher concentrations of 10^{-5} to 3×10^{-5} M this parallelism disappeared and there was a marked decrease in the maximum response, indicating that the antagonism between desipramine and NA was not competitive (Figure 3c). With desipramine at 3×0^{-5} M the pD'_2 value was 4.68 ± 0.08 (6). Desipramine also had inhibitory activity against ACh. The concentration-response

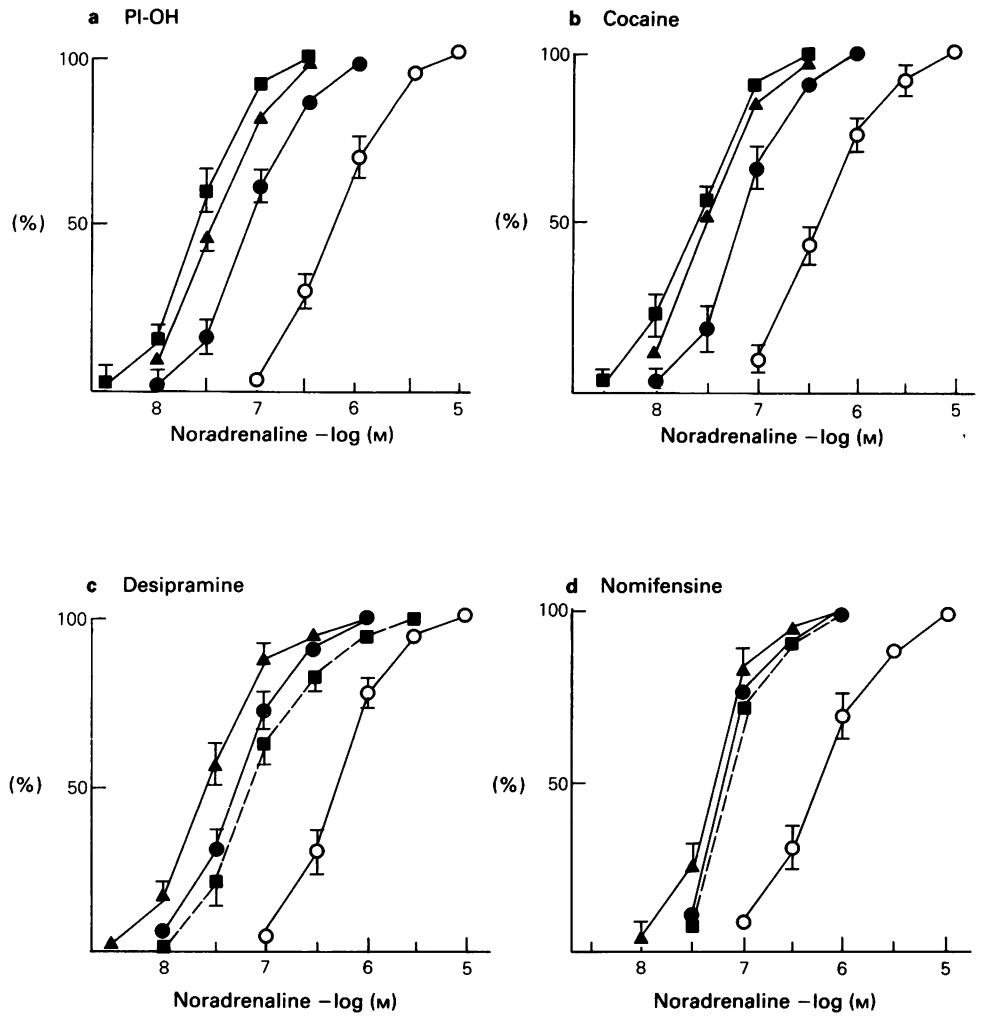

Figure 2 Log concentration-response curves to noradrenaline (NA) in the presence of 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH) (a), cocaine (b), desipramine (c) and nomifensine (d) of rat anococcygeus muscle. Responses are expressed as percentage of the maximal control response to NA. Concentration-response curves to NA in the absence of each test drug are shown by open circles and those in the presence of each test drug by various closed symbols. (a) Responses in absence (○) and presence of PI-OH, 3×10^{-7} M (●), 10^{-6} M (▲) and 3×10^{-6} M (■). (b) Responses in absence (○) and presence of cocaine, 10^{-6} M (●), 3×10^{-6} M (▲) and 10^{-5} M (■). (c) Responses in absence (○) and presence of desipramine , 3×10^{-7} M (●), 10^{-6} M (▲) and 3×10^{-6} M (■). (d) Responses in absence (○) and presence of nomifensine, 3×10^{-7} M (●), 10^{-6} M (▲) and 3×10^{-6} M (■). Note that the curves shown by dotted lines in the presence of 3×10^{-6} M desipramine and nomifensine indicate inhibition relative to those in their presence at 10^{-6} M. Points and bars represent means and s.e. mean for 9 experiments for PI-OH and 6 experiments for other drugs.

curves of ACh were shifted in parallel to the right in the presence of desipramine at 3×10^{-6} and 10^{-5} M, but at 3×10^{-5} M the maximum response to ACh was greatly depressed (Figure 3d). Desipramine at 3×10^{-6} M and 10^{-5} M, caused competitive antagonism against ACh and gave a pA_2 value of 6.18 ± 0.08 (6) and a slope of 1.08 ± 0.06 (6). But, when its concentration was increased to 3×10^{-5} M, the pD'_2 value was 4.85 ± 0.05 (6), indicating non-competitive activity. Nomifensine and PI-OH at up to 10^{-5} M did not show any inhibitory activity against ACh.

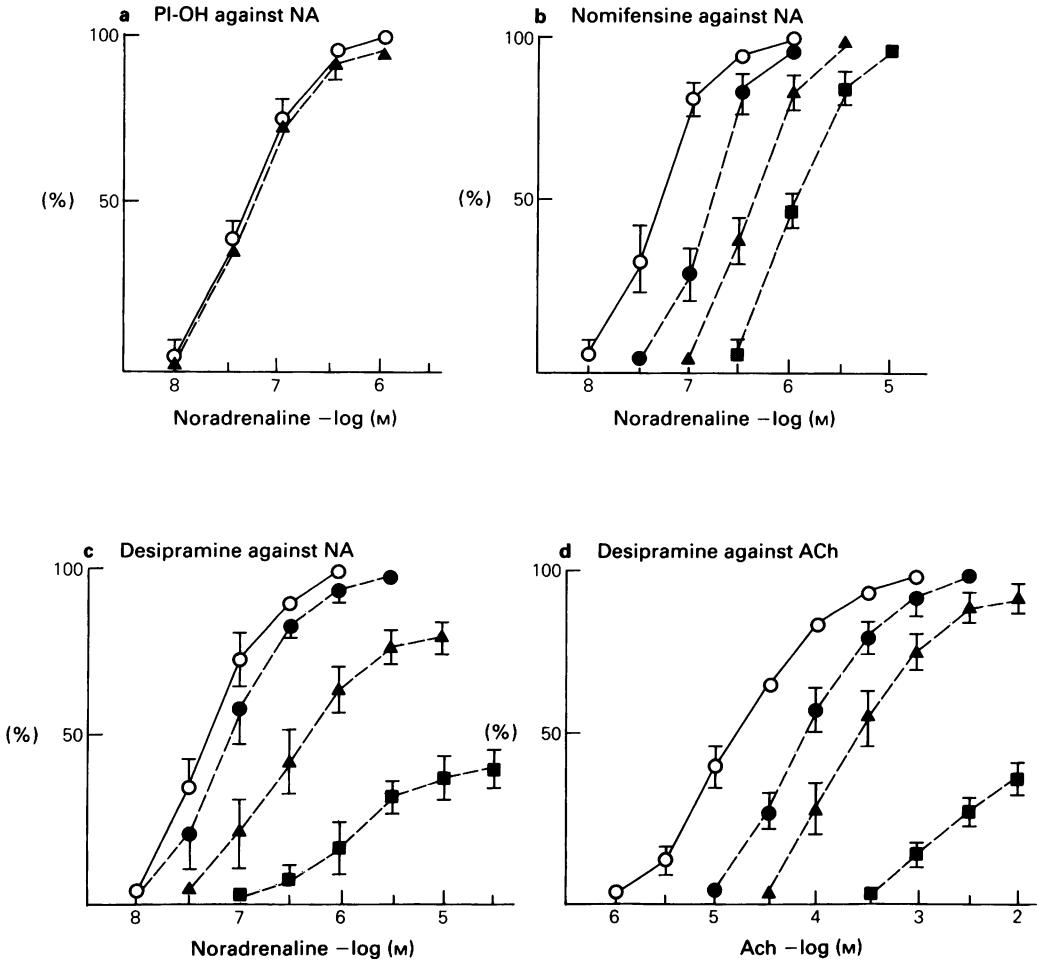

Figure 3 Postsynaptic inhibitory effects of 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH) (a), nomifensine (b) and desipramine (c and d) on rat anococcygeus muscles. The effects of the three drugs are shown in comparison with control noradrenaline (NA) curves in the presence of cocaine 10^{-5} M and propranolol 10^{-7} M. The concentrations of test drugs are as follows: 3×10^{-6} M (●), 10^{-5} M (▲) and 3×10^{-5} M (■). Note that PI-OH (a) has no inhibitory effect, but that nomifensine (b) and desipramine (c) cause competitive and non-competitive inhibition against NA, respectively. Desipramine also shows antagonistic activity against ACh in a competitive and non-competitive manner (d). Points and bars represent means and s.e. mean for 6 experiments.

These results indicate that desipramine and nomifensine have non-competitive and competitive inhibitory effects on postsynaptic α-adrenoceptors, respectively. But PI-OH has no postsynaptic inhibitory effect on the response to NA at concentrations up to 10^{-5} M.

Effects of drugs on nerve stimulation

Electrical field stimulation of the rat anococcygeus muscle produces twitch contractions due to adrenergic innervation of the muscle, because the response was blocked by tetrodotoxin (10^{-6} M), prazosin (10^{-6} M) or guanethidine (10^{-5} M).

Increasing concentrations of PI-OH gradually potentiated the control twitch contractions (27.0% of the NA maximum contraction) induced by field stimulation and the potentiation by PI-OH at 10^{-6} M was 85.4% (Figure 4a and Table 2). At higher concentrations of 3×10^{-6} and 10^{-5} M, PI-OH showed no inhibitory effect on these potentiated twitch contractions. Cocaine also potentiated the twitch contractions, but its activity was weaker than

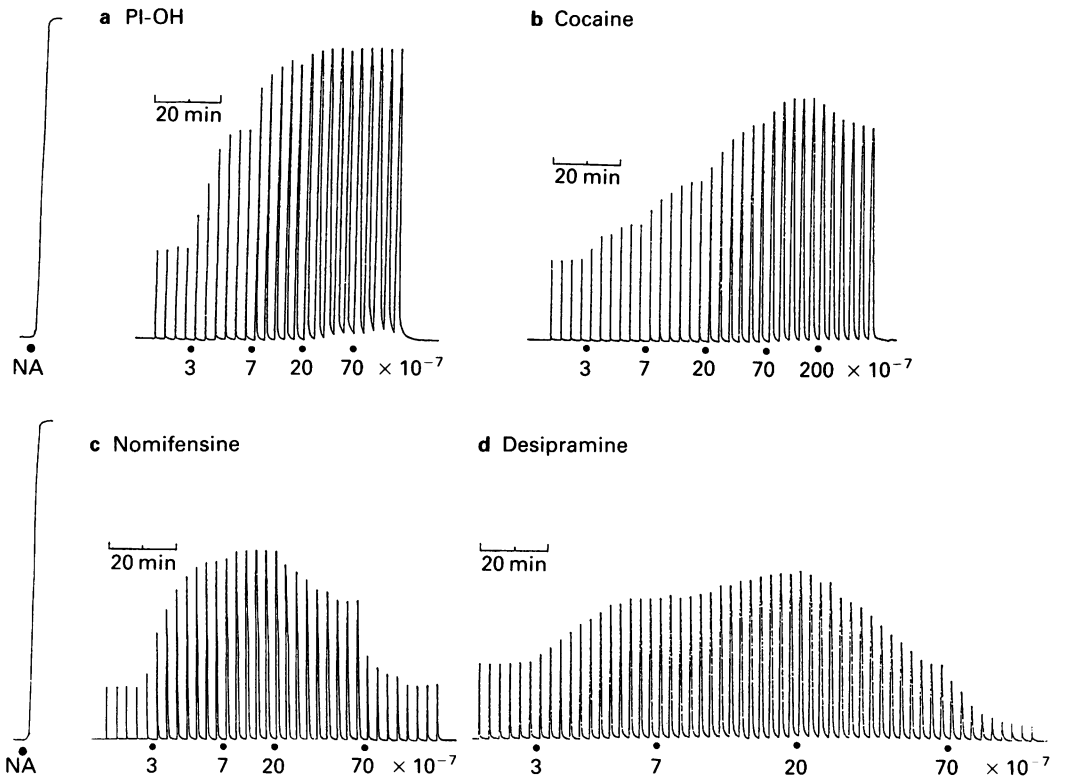

Figure 4 Examples of the effects of 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolone (PI-OH) (a), cocaine (b), nomifensine (c) and desipramine (d) on the response of rat anococcygeus muscle to field stimulation. The muscle was stimulated at 5 Hz for 0.5 or 1 ms. Maximal contractions induced by noradrenaline (NA, 10^{-5} M) are shown on the left.

that of PI-OH. Its potentiation was maximal at 10^{-5} M and was 77.1% of the NA maximum. Both nomifensine and desipramine at low concentrations of 3×10^{-7} and 10^{-6} M caused potentiation, but at high concentrations of 3×10^{-6} and 10^{-5} M inhibitory effects on twitch contractions gradually appeared. Examples of the effects of nomifensine and desipramine are illustrated in Figure 4c and d. The means for data obtained in 6 experiments with each drug are shown in Table 2.

Discussion

Nash *et al.* (1974) showed that at NA concentrations below $2\,\mu g\,ml^{-1}$ the rat anococcygeus muscle accumulates NA mainly into adrenergic nerves, with little contribution by extraneural uptake ($Uptake_2$). The increase produced by cocaine or many antidepressants in the sensitivity of the anococcygeus muscle to NA was consistent with the view that these drugs cause supersensitivity in adrenergically-innervated tissues by inhibiting the neural uptake mechanism (Trendelenburg, 1966).

The chemical structure of PI-OH is similar to that of nomifensine, which is a non-tricyclic antidepressant (Hoffman, 1973). Nomifensine produces dopamine-like symptoms in the rat, which were stated to be related to the dopamine releasing properties of the drug (Braestrup & Scheel-Krüger, 1976). More recent studies (McKillop & Bradford, 1981) suggested that nomifensine blocks dopamine uptake into striatal synaptosomes, and that it also inhibits release of dopamine, due to an agonist effect on presynaptic dopamine receptors. However, there are no reports showing a potentiating action of NA on sympathetically innervated isolated tissues so it is important to compare the relative activity against NA potentiation of nomifensine with that of PI-OH.

Many antidepressants including desipramine have a dual effect on the response of peripheral adrenergically-innervated tissues to either nerve

Table 2 Potentiating activities of test drugs on the response to electrical field stimulation of rat anococcygeus muscle

Test drugs	*Control*[a]	*Concentrations of test drugs* 3×10^{-7} M	10^{-6} M	3×10^{-6} M	10^{-5} M
PI-OH	27.0 ± 2.5%	67.3 ± 6.2%	85.4 ± 4.2%	87.4 ± 2.5%	85.7 ± 2.6%
Cocaine	24.7 ± 1.2%	33.2 ± 4.0%**	54.2 ± 4.7%**	69.3 ± 5.5%*	77.1 ± 4.3%
Nomifensine	21.5 ± 3.0%	64.7 ± 3.7%	65.7 ± 3.3%**	54.4 ± 3.2%**	23.6 ± 2.6%**
Desipramine	22.4 ± 2.6%	36.1 ± 2.2%**	41.9 ± 5.7%**	29.1 ± 5.9%**	5.3 ± 2.4%**

[a] Values are percentages of the noradrenaline (NA) maximal contraction and are means ± s.e. mean of 6 experiments.
[b] The asterisks indicate the levels of significance (unpaired t test) when compared with the value for 4-hydroxy-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (PI-OH) at each concentration (* $P < 0.05$; ** $P < 0.01$).

stimulation or exogenous NA (Hrdina & Ling, 1970; Doggrell & Woodruff, 1977). At low concentrations they potentiate the responses to both nerve stimulation and exogenous NA administration. The potentiation of NA-induced responses by antidepressants is believed to result from impaired uptake of NA into adrenergic nerve endings so that a greater concentration of NA is made available at the receptor sites. Then, as the concentration of the drugs is increased this potentiating effect becomes progressively masked by an inhibitory one, presumably because the adrenolytic activity of the drugs becomes significant in higher concentrations (Doggrell & Woodruff, 1977). However, the postsynaptic inhibitory action of desipramine is not selective and is not restricted to those cases in which contraction is produced by α-adrenoceptor-stimulating drugs on the rat renal artery (Hrdina & Ling, 1970). Our data on the rat anococcygeus muscle showed that desipramine was an antagonist with non-selective actions which may be due to a calcium antagonist action as reported by Hrdina & Garattini (1967), because responses to both NA and ACh were inhibited by the presence of desipramine (Figure 3c and d). Desipramine also has an anti-histaminic effect on guinea-pig isolated ileum with a pA_2 value of 7.23 and slope of 0.97 (unpublished results). The postsynaptic inhibitory action of nomifensine seems to be selective for α-adrenoceptors, because after pretreatment of rat anococcygeus muscle with nomifensine, the concentration-response curves of NA are shifted in parallel to the right without decrease in the maximum, but the concentration-response curves of ACh are not shifted to the right. PI-OH at up to 10^{-5} M did not cause any postsynaptic inhibition of the responses of the muscle to NA and ACh. It is therefore understandable that PI-OH at concentrations of 3×10^{-7}, 10^{-6} and 3×10^{-6} M produces progressive potentiation of the NA concentration-response curve or of contractions induced by field stimulation without any inhibitory effect at higher concentrations. PI-OH had a similar potentiating effect to that of cocaine, but its activity was greater than that of cocaine at all concentrations tested. Desipramine and nomifensine had stronger potentiating effects on the NA concentration-response curve at a low concentration of 3×10^{-7} M, but at a higher concentration of 3×10^{-6} M their activity was less than that of PI-OH (Table 1). On electrical stimulation, PI-OH had the strongest potentiating activity of the compounds tested, even at a low concentration of 3×10^{-7} M (Table 2). Both nomifensine and desipramine at low concentrations of 3×10^{-7} M and 10^{-6} M potentiated the twitch contractions induced by field stimulation, but at higher concentrations their effect changed progressively to inhibition (Figure 4).

Kenakin & Beek (1981) have presented a caveat on indiscriminate use of uptake inhibitors to achieve equilibrium conditions in isolated tissue experiments. Their theoretical calculations indicate that more than 20 fold selectivity of the uptake-inhibitor for the site of uptake over the receptor is required to ensure no interference in the measurement of antagonist potency and the data with amitriptyline and metanephrine are insufficient. Our data indicate that a new potentiator of NA, PI-OH may be an ideal potentiator for the NA uptake, because it has no side effects such as inhibitory actions against the postsynaptic receptors and the muscle itself. Moreover, the potentiating activity of PI-OH for the NA response of the anococcygeus muscle was stronger than that of cocaine. However, the effects of nomifensine and desipramine on the response of the tissues to NA were a balance between potentiation due to block of uptake and antagonism due to postsynaptic inhibitory actions. The antagonistic action of nomifensine against NA was competitive while that of desipramine was non-competitive.

Our thanks are due to Hoechst, Japan for a gift of nomifensine and Misses T. Kondo, M. Toyama and S. Ono for excellent technical assistance.

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(Received June 3, 1987
Revised November 9, 1987
Accepted December 21, 1987)