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SELECTIVE POTENTIATION OF NORADRENALINE IN THE GUINEA-PIG VAS DEFERENS BY 2-(4-METHYLAMINOBUTOXY) DIPHENYLMETHANE HYDROCHLORIDE (MCI-2016), A NEW PSYCHOTROPIC DRUG

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1 In the isolated vas deferens of the guinea-pig, the effects of 2-(4-methylaminobutoxy) diphenylmethane hydrochloride (MCI-2016), a new psychotropic drug, on the contractile response to various agonists or transmural electrical stimulation and on the release of noradrenaline (NA) from the tissue were examined and compared with cocaine.

2 MCI-2016 (3×10^{-6} M) and cocaine (3×10^{-5} M) produced a leftward shift (15 and 20 times, respectively) of the dose-response curves for the contractile effect of NA and increased the maximum contractile response to NA by approximately 7 and 14% respectively.

3 MCI-2016 had no apparent effect on the dose-response curves for methoxamine, acetylcholine and high K, while cocaine markedly shifted those for these agents to the left and increased the maximal responses (10, 16 and 16%, respectively).

4 MCI-2016 and cocaine abolished the tyramine (3×10^{-4} M)-induced contraction and inhibited the twitch response to transmural electrical stimulation in a dose-dependent manner. The inhibitory effects of both drugs on the twitch were reversed by yohimbine (10^{-5} M).

5 The spontaneous outflow of NA from the vas deferens was unaffected by MCI-2016 (3×10^{-6} M) and cocaine (10^{-5} M), while the high-K-evoked release of NA was increased by both drugs.

6 In the presence of cocaine (10^{-5} M), the high-K-evoked release of NA was markedly increased by yohimbine (10^{-5} M) and decreased by clonidine (3×10^{-8} M), but only slightly increased by MCI-2016 (3×10^{-6} M).

7 In phaeochromocytoma cells, both MCI-2016 and cocaine at concentrations of 10^{-7} to 10^{-5} M caused a dose-dependent inhibition of the [^{3}H]-NA uptake.

8 These results suggest that MCI-2016-induced supersensitivity is specific for NA and is due to interference with the neuronal uptake process for NA.

Introduction

2-(4-Methylaminobutoxy) diphenylmethane hydrochloride (MCI-2016), one of the (ω-aminoalkoxy) benzene derivatives was found to possess potent antidepressive activity (Kikumoto, Tobe & Tonomura, 1981). Recently it has been revealed that MCI-2016 markedly antagonizes the hypothermia and depression-like syndrome induced by reserpine and exhibits such activities as antitetrabenzine and anticataleptic actions (Tobe, Yoshida, Ikoma, Tonomura & Kikumoto, 1981). Anticholinergic and sedative actions of MCI-2016 were considerably weaker than those of tricyclic antidepressants such as amitriptyline and imipramine which have been used for the therapy of depremention (Tobe *et al.*, 1981). Preliminary experiments indicated that MCI-2016 potentiated the contractile response of the rat vas deferens to noradrenaline (NA). Therefore, the present experiments were undertaken to clarify its potentiation mechanism in the guinea-pig isolated vas deferens; its effects were compared with those of cocaine.

Methods

Mechanical response

The vasa deferentia were removed from male guinea-pigs weighing 250 to 350 g. The preparation of the vas deferens and technique for measurement of the response was carried out as described previously (Ohizumi & Shibata, 1980). Tissues were suspended in a 20 ml organ bath containing Krebs solution of the following composition (mM): NaCl 120, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.2

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and gassed with 95% O_2 and 5% CO_2 and temperature was maintained at 32°C. The vas deferens was stimulated transmurally with rectangular pulses of 1 ms duration and supramaximal voltage. The stimuli were applied for periods of 1 s every 20 s. NA, methoxamine, acetylcholine (ACh) and high-K (50 mM) were applied to the bath medium 10 min after MCI-2016 or cocaine was applied.

Assay of noradrenaline released from the vas deferens

Guinea-pigs weighing 300 to 350 g were used. The vas deferens was placed in a bath containing Krebs solution (4 ml) which was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. When the effect of MCI-2016 or cocaine on the resting outflow of NA was measured, the tissues were preincubated for 60 min, during which time the solution was changed every 15 min. Then the tissues were transferred to the normal Krebs solution or the normal Krebs solution containing MCI-2016 or cocaine for 30 min. Control tissues were exposed to a fourth period of 15 min in Krebs solution, to parallel the 15 min exposure period of the test tissues to the Krebs solution containing various drugs. Finally control tissues were transferred to the normal solution containing high-K for 30 min, to parallel the 30 min exposure period of the test tissues to normal solution containing high-K and each drug. Chemical determination of NA released from the vas deferens into the incubation medium was carried out as described previously (Ohizumi & Shibata, 1980).

Assay of [^{3}H]-noradrenaline uptake into phaeochromocytoma (PC12h) cells

[^{3}H]-NA uptake assay was performed as described by Greene & Rein (1977). PC12h cells, which were established by Greene & Tischler (1976) and subcloned by Hatanaka (1981), were grown at 37°C in a glass culture flask containing Dulbecco's modified Eagle's medium supplemented with 5% (v/v) heat inactivated horse serum and 5% (v/v) new born calf serum. The cells were harvested 24 h before experiments, and plated on polylysine-coated 35 mm Falcon tissue culture dishes in the same medium. After removal of the growth medium, the cells were preincubated at 37°C for 15 min in 1 ml of the assay solution of the following composition (mM): NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgSO_4$ 0.8, NaH_2PO_4 1, glucose 5.5, HEPES 50 and pargyline 0.1; pH 7.4. After removal of the preincubated solution, 1 ml of the assay solution containing [^{3}H]-NA (5×10^{-6} M; sp. act. 0.2 Ci/mmol) was added to each dish. After 10 min incubation, the solution was removed and the cells were washed quickly five times with the ice-cold solution. The cells were then scraped from the dish and dissolved in 0.4 ml of 0.25 N NaOH and incubated at 60°C for 20 min; 0.2 ml of this extract were transferred to a scintillation counting vial containing 10 ml scintillation mixture and its radioactivity counted. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as the standard.

Statistical analysis of the data

Mean data on mechanical response and chemical assay of NA are presented with the standard error of the mean (s.e.mean) and Student's t test was used to evaluate the results in all the experiments with n as the number of experiments.

Drugs and chemicals

The following drugs and chemicals were used in the present study. MCI-2016 was synthesized as described previously (Kikumoto *et al.*, 1981). The chemical structure of MCI-2016 is shown in Figure 1. Other agents included: cocaine hydrochloride (Takeda Yakuhin Co.), noradrenaline bitartrate (Sigma Co.), acetylcholine chloride (Daiichi-Seiyaku Co.), methoxamine hydrochloride (Burroughs Wellcome Co.), tyramine hydrochloride (Sigma Co.), yohimbine hydrochloride (Wako Pure Chemical Co.) clonidine hydrochloride (Tokyo-Kasei Co.), pargyline (Sigma Co.), HEPES (Sigma Co.) (−)-[ring-2,5,6-^{3}H]-noradrenaline (New England Nuclear Co.) Dulbecco's modified Eagles medium (GIBCO), horse serum (GIBCO), new born calf serum (Mitsubishi-Kasei Co.) and bovine serum albumin (Sigma Co.).

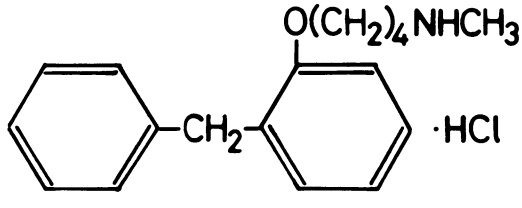

Figure 1 Structural formula of 2-(4-methylaminobutoxy) diphenyl-methane hydrochloride (MCI-2016).

Results

Mechanical response

In the guinea-pig vas deferens, MCI-2016 at concentrations of 3×10^{-7} to 3×10^{-6} M and cocaine at a concentration of 3×10^{-6} M had no effect alone but caused a leftward shift of the dose-response curve for

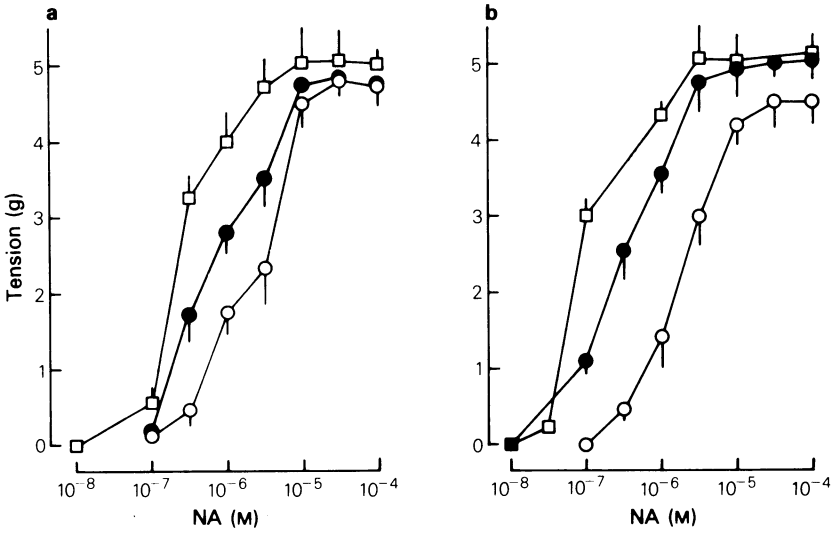

Figure 2 Effects of MCI-2016 (a) and cocaine (b) on the dose-response curves for noradrenaline (NA) in the guinea-pig vas deferens: (a) (○) control; (●) MCI-2016 3×10^{-7} M; (□) MCI-2016 3×10^{-6} M. In (b), (○) control; (●) cocaine 3×10^{-6} M; (□) cocaine 3×10^{-5} M. MCI-2016 and cocaine were added 10 min before application of NA. Each point and bar represents the mean and s.e.mean of 7 experiments.

the contractile effect of NA and an increase in the maximal responses, indicating supersensitivity (Figure 2). With MCI-2016 3×10^{-7} M and 3×10^{-6} M, the leftward shifts were 5 and 15 times, respectively, and the maximal responses were increased by approximately 3 and 7%, respectively, while cocaine (3×10^{-6} and 3×10^{-5} M) also produced a leftward shift (approximately 7 and 20 times, respectively) and increased the maximal responses (approximately 11 and 13%, respectively). On the other hand, MCI-2016 (3×10^{-6} M) did not affect the leftward shift of the dose-response curves for methoxamine, acetylcholine (ACh) and high K or the maximal responses (Figure 3). Cocaine (3×10^{-6} M) produced slight

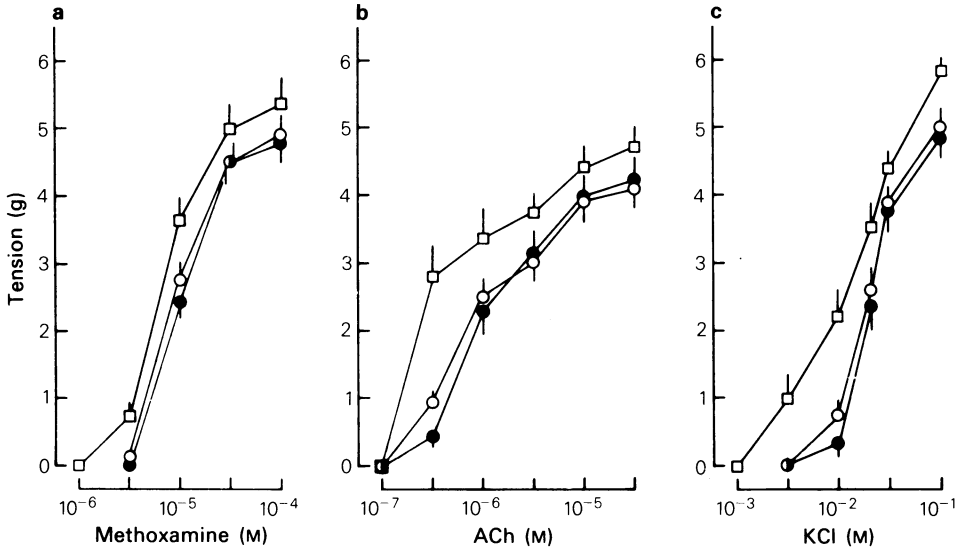

Figure 3 Effects of MCI-2016 and cocaine on the dose-response curves for methoxamine (a), acetylcholine (ACh) (b) and high-K (c) in the guinea-pig vas deferens: (○) control; (●) MCI-2016 3×10^{-6} M; (□) cocaine 3×10^{-5} M. MCI-2016 and cocaine were added 10 min before methoxamine, ACh or high K. Each point and bar represents the mean and s.e.mean of 7 experiments.

shifts of the dose-response curves for methoxamine, ACh and high K and the maximal responses were increased by approximately 10, 16 and 16%, respectively (Figure 3). In addition, doses of MCI-2016 higher than 3×10^{-5} M, showed inhibitory effects on the contractile response to these agonists.

Both MCI-2016 (3×10^{-6} M) and cocaine (10^{-5} M) abolished the tyramine (3×10^{-4} M)-induced contraction (2.2 ± 0.2 g). The contractile response (twitch) to transmural electrical stimulation (3 Hz, 1 ms, supramaximal voltage) was inhibited by MCI-2016 (10^{-8}–3×10^{-6} M) and cocaine (10^{-7}–10^{-5} M) in a dose-dependent manner. The inhibitory effects of MCI-2016 (3×10^{-6} M) and cocaine (10^{-5} M) on the twitch were antagonized by yohimbine (10^{-5} M), whereas those of tetracaine (10^{-5} M) were unaffected by yohimbine (Figure 4).

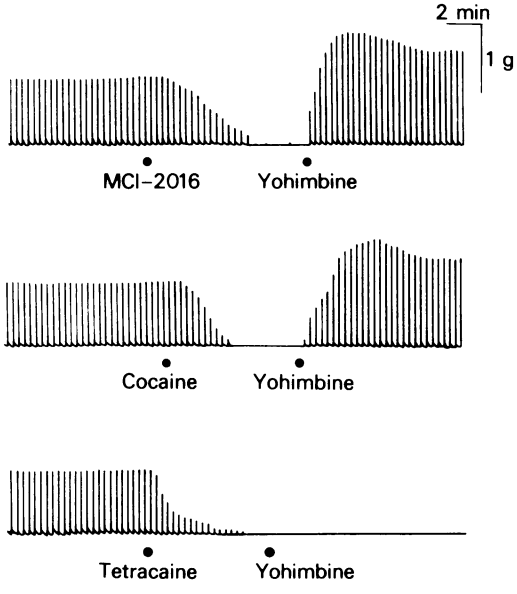

Figure 4 Effects of MCI-2016 and cocaine on the contractile response of the guinea-pig vas deferens to transmural stimulation (3 Hz, 1 ms, supramaximal voltage) which was applied for 1 s every 20 s. MCI-2016 (3×10^{-6} M), cocaine (10^{-5} M), tetracaine (10^{-5} M) and yohimbine (10^{-5} M) were added at ●.

Assay of released noradrenaline

The spontaneous outflow of NA from the guinea-pig vas deferens was unaffected by treatment with MCI-2016 or cocaine at any concentrations from 10^{-7} to 10^{-5} M. Table 1 shows the effect of various drugs on the high-K (50 mM)-evoked release of NA from the vas deferens. The high-K-evoked release of NA was increased to approximately 290% in the presence of MCI-2016 (3×10^{-6} M) or cocaine (10^{-5} M). In the presence of cocaine (10^{-5} M), the high-K-evoked release of NA was increased to approximately 141% by yohimbine (10^{-5} M) and decreased to approximately 49% by clonidine (3×10^{-8} M), but increased to only 108% by MCI-2016 (3×10^{-6} M).

Assay of [^{3}H]-noradrenaline uptake

Figure 5 shows the inhibitory effect of MCI-2016 and cocaine on the uptake of [^{3}H]-NA into PC12h cells. The amount of [^{3}H]-NA taken up in the absence of agents was 35 pmol min^{-1} mg^{-1} protein. Both MCI-2016 and cocaine at concentrations of 10^{-7} to 10^{-5} M caused a dose-dependent inhibition of the [^{3}H]-NA uptake. ID_{50}s of both agents were estimated as approximately 3×10^{-6} M.

Table 1 Effect of MCI-2016 and cocaine on the release of noradrenaline (NA) from the guinea-pig vas deferens

Treatment	*Amount of NA released*
None	40.1 ± 5.3
KCl (50 mM) (Control)	72.0 ± 12.5
KCl (50 mM) + MCI-2016 (3×10^{-8} M)	83.8 ± 12.1
KCl (50 mM) + MCI-2016 (3×10^{-7} M)	190.1 ± 23.5*
KCl (50 mM) + MCI-2016 (3×10^{-6} M)	210.3 ± 7.3*
KCl (50 mM) + Cocaine (10^{-5} M)	209.3 ± 33.4*
KCl (50 mM) + Cocaine (10^{-5} M) + MCI-2016 (3×10^{-6} M)	225.2 ± 39.6*
KCl (50 mM) + Cocaine (10^{-5} M) + Yohimbine (10^{-5} M)	294.0 ± 29.3
KCl (50 mM) + Cocaine (10^{-5} M) + Clonidine (3×10^{-8} M)	103.5 ± 15.3

High K was applied 15 min after treatment with MCI-2016, cocaine, cocaine + MCI-2016, cocaine + yohimbine and cocaine + clonidine. Measurements were made 30 min after application of high K. Five to six preparations were used for each experiment.

*Significantly different from control ($P < 0.01$).

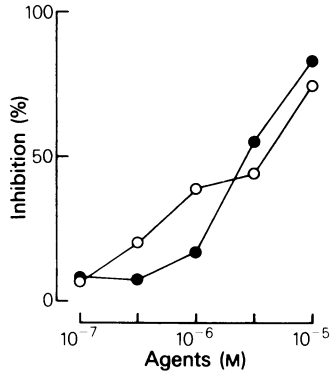

Figure 5 Dose-response curves of inhibitory effects of MCI-2016 (○) and cocaine (●) on the uptake of [^{3}H]-noradrenaline ([^{3}H]-NA) into PC12h cells. PC12h cells were incubated with [^{3}H]-NA (5×10^{-6} M) in the presence or absence of various concentrations of MCI-2016 and cocaine for 10 min at 37°C.

Discussion

In the guinea-pig vas deferens, MCI-2016 markedly shifted the NA dose-response curves to the left, but had no effects on those for methoxamine, an α-adrenoceptor agonist which is not taken up by adrenergic nerve endings (Trendelenburg, Maxwell & Pluchino, 1970). Furthermore, the dose-response curves for ACh and high K were almost unaffected in the presence of MCI-2016. On the other hand, cocaine produced a marked leftward shift of the dose-response curves for NA and slightly shifted those for methoxamine, ACh and high K. These results indicate that the MCI-2016-induced supersensitivity is specific for NA, while that of cocaine has a nonspecific component.

Cocaine has been shown to block the NA reuptake mechanism of adrenergic nerves in various smooth muscle preparations (Trendelenburg, 1963; 1969). In the present experiments, MCI-2016 caused a specific potentiation of NA and, like cocaine, abolished the contractile response of the vas deferens to tyramine, which is taken up into adrenergic nerve terminals to release endogenous NA (Trendelenburg, Muskus, Fleming & Gomerz & Sierra, 1962; Muscholl, 1966). The high-K-evoked release of NA from the vas deferens was greatly increased by both MCI-2016 and cocaine. However, MCI-2016, but not yohimbine, failed to cause a further increase in the amount of NA released by high K when neuronal uptake of NA was already inhibited by cocaine. These results suggest that MCI-2016 increased the high-K-evoked NA release by inhibiting the uptake process of NA, but not by blocking presynaptic α-adrenoceptors. Since it has been demonstrated that PC12 cells are useful model systems for studying catecholamine metabolism in adrenergic nervous system (Greene & Tischler, 1976; Greene & Rein, 1977), the assay of [^{3}H]-NA uptake into PC12h cells indicated that MCI-2016 has indeed potent inhibitory effects on neuronal uptake of [^{3}H]-NA.

It is now widely accepted that the release of NA from adrenergic nerve endings is regulated by a negative feedback mechanism through the activation of presynaptic α-adrenoceptors (Langer, 1977; Starke, 1977; Westfall, 1977). Recently, cocaine has been shown to reduce electrically-induced contractions of the vas deferens (Starke, Borowski & Endo, 1975; Marshall, Nasmyth & Shepperson, 1977). Also, it was previously found that the inhibitory effect of cocaine was reversed in the presence of yohimbine, a presynaptic α-adrenoceptor agonist (Marshall *et al.*, 1977). In the present experiments, MCI-2016, like cocaine, inhibited the twitch induced by transmural stimulation. Yohimbine caused complete reversal of the inhibition by both drugs, but did not reverse that produced by tetracaine, a powerful local anaesthetic, probably indicating that MCI-2016 and cocaine, at the concentrations used, inhibit the twitch response by a mechanism not involving a local anaesthetic action. Together these observations lead us to speculate that the twitch inhibition by MCI-2016 may be mainly attributed to activation of presynaptic α-inhibitory feedback system caused by an accumulation of endogenous NA.

On the basis of these data, it is concluded that MCI-2016-induced supersensitivity in the vas deferens is almost entirely prejunctional and is due to interference with the neuronal uptake process for NA. It is also suggested that cocaine-induced supersensitivity has both pre- and postjunctional components.

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