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Characterization of α_1-adrenoceptor subtypes in facilitation of rat spinal motoneuron activity

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Abstract

The subtypes of α_1-adrenoceptor mediating facilitation of α-motoneuron activity in adult rat spinal cord were examined. The potencies of agonists and antagonists were compared, using extracellular single unit recordings made in spinal cord slices isolated from adult rats. Catecholamines (epinephrine, norepinephrine), phenylethylamines (phenylephrine, methoxamine) and imidazolines (clonidine, oxymetazoline, St587 (2-(2-chloro-5-trifluoromethyl–phenylimino)-imidazoline)) enhanced motoneuron activity. Catecholamines and phenylethylamines elicited maximal responses but imidazolines elicited submaximal responses. The potencies of the agonists were similar to their affinities for α_{1A}-adrenoceptors. The α_{1A}-adrenoceptor agonist SDZ NVI 085 ((−)-(4a*R*,10a*R*)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth[2,3b]-1,4-oxazine) behaved as a partial agonist with a maximal response of about 75% and this response was not inhibited by chloroethylclonidine. Pretreatment with chloroethylclonidine produced a rightward shift of the phenylephrine response curve but caused little reduction in the maximal response. Prazosin, 5-methyl-urapidil, WB4101 (2-([2,6-dimethoxyphenoxyethyl] aminomethyl)-1,4-benzodioxane) and BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) concentration dependently inhibited the facilitation of α-motoneuron activity produced by phenylephrine. The order of inhibitory potency was similar to that for their α_{1A}-adrenoceptor binding site affinity. Therefore, it seems that α_{1A}-adrenoceptors may be functionally predominant in the facilitation of rat spinal motoneuron activity. © 1997 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor subtype; Motoneuron; Spinal cord slice; (Rat, adult)

1. Introduction

Descending noradrenergic fibers originating from the brainstem nuclei and terminating in the spinal ventral horn play an important role in controlling motoneuron activity. It has been shown that L-DOPA has effects on spinal reflex transmission and that the actions of L-DOPA are blocked by α-adrenoceptor blockers (Andén et al., 1966). Noradrenaline has been shown to produce an increased excitability of rat motoneurons (McCall and Aghajanian, 1979). Conway et al. (1988) demonstrated that L-DOPA can induce plateau potentials in cat motoneurons. These facilitations of motoneuron activity are induced via α_1-adrenoceptors. Our previous studies of the spinal reflex, decerebrate rigidity and spinal cord slices of rats support this idea (Ono and Fukuda, 1995).

Recently, evidence has been reported that there are subtypes of the α_1-adrenoceptor, but there are few reports about these subtypes in the spinal cord. The initial subdivision of the α_1-adrenoceptors of the rat spinal cord into the α_{1A}- and α_{1B}-adrenoceptor classes was based on the different affinity of a competitive antagonist, WB4101 (2-([2,6-dimethoxyphenoxyethyl] aminomethyl)-1,4-benzodioxane), and on the different sensitivity to the irreversible alkylating agent, chloroethylclonidine (Wilson and Minneman, 1989). These experiments showed that α_{1A}- and α_{1B}-adrenoceptors are heterogeneously distributed in rat brain and that the α_{1A}-adrenoceptor is predominant in rat cervical spinal cord. The in vivo experiment of Bervoets and Millan (1994) suggested that spinal α_{1A}-adrenoceptors mediate the spontaneous tail flicks induced by 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); however, it is not clear whether this response is directly induced in the spinal motoneurons or indirectly induced in other neurons.

Recently, three native α_1-adrenoceptors and three recombinant α_1-adrenoceptors were identified. The relationship between native and recombinant α_1-adrenoceptor sub-

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types has been determined and a nomenclature to identify them has been recommended (Hieble et al., 1995). Following this nomenclature, we previously characterized the subtypes of α_1-adrenoceptors in rat spinal lumbar cord (Wada et al., 1996), and found α_{1A}- and α_{1B}-adrenoceptor populations to account for 70% and 30%, respectively, of the total and that very few α_{1D}-adrenoceptors are present. Although the distribution of the subtypes of α_1-adrenoceptors is known, the subtypes of α_1-adrenoceptor which mediate facilitation of α-motoneuron activity in the rat lumbar spinal cord have not been characterized. Therefore, we investigated the subtypes of α_1-adrenoceptors which are functionally predominant. In the present study, we characterized the subtypes of α_1-adrenoceptor mediating facilitation of α-motoneuron activity by using agonists and competitive and non-competitive antagonists with isolated spinal cord slice preparations from adult rats.

We provide evidence for the characterization of α_1-adrenoceptor subtypes in the motoneurons of the rat lumbar spinal cord and this is one of the few studies of α_1-adrenoceptor subtypes to use electrophysiological procedures.

2. Materials and methods

2.1. Single-unit recording

The details of the procedure have been reported previously (Hirayama et al., 1988a,b). Male Wistar rats, aged about 7 weeks, were anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.). Following laminectomy, slices of about 430 μm thickness were obtained by sectioning transverse to the longitudinal axis of the spinal cord, using a tissue slicer (Dosaka EM, DTK-2000). The slices were incubated in artificial cerebrospinal fluid at 33°C for more than 1 h. Each slice was transferred to a 1-ml capacity recording chamber and continuously perfused with artificial cerebrospinal fluid at a rate of 2.5 ml/min. The artificial cerebrospinal fluid consisted of (in mM), NaCl 124, KCl 5, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, KH_2PO_4 1.24 and glucose 10, and was saturated with 95% O_2, 5% CO_2 (pH 7.4). The perfusion medium was kept at 33°C and contained 0.1 μM desipramine. Electrical stimulation, consisting of single rectangular pulses (frequency 1/6 s, duration 0.05 ms, SEN-3301, Nihon Kohden), was applied by means of bipolar tungsten electrodes placed near the motor nuclei of the ventral horn. Extracellular unit activity was recorded close to the site of stimulation in the motor nuclei of the ventral horn, using a glass microelectrode filled with 4 M NaCl (1–3 MΩ). Unitary action potentials were displayed on a memory oscilloscope (ATAC-350 Nihon Kohden) after first passing through a high-input-impedance preamplifier (MEZ-8300, Nihon Kohden). Any stimulus artifacts in the responses were omitted from the memory and were recorded on a thermal array recorder (WR7400, Graphtec). Typical data are shown in Fig. 1A. Several cells responded with a short latency and a high-frequency burst discharge. These responses are derived from interneurons such as Renshaw cells (Hirayama et al., 1988b). We did not use these cells but used the cells in which only a single action potential was observed, with a latency of 1–2 ms. The threshold of firing was defined as the stimulus intensity that just failed to discharge an action potential in response to each stimulus. The stimulation voltage was adjusted so that the cell discharged at a low probability (less than 10–20%) of less than once or twice per 10 stimuli, so that the excitatory effects produced by α_1-adrenoceptor agonists could be observed.

2.2. Chloroethylclonidine treatment

Slices of rat spinal cord were treated with chloroethylclonidine for a total of 30 min; 15 min after its initial application, chloroethylclonidine was reapplied to avoid decomposition.

2.3. Data analysis

All results are shown as the means ± S.E.M.. Dose–response curves were fitted to a standard sigmoid curve, using DeltaGraph Pro (DeltaPoint). Agonist pD_2 values were determined by calculating the negative logarithms of the EC_{50} values. Antagonist pA_2 values were determined from Schild plots.

2.4. Drugs

The drugs used were BMY7378 (8-[2-[4-(2-methoxyphenyl)-1- piperazinyl]ethyl]-8-azaspiro[4.5] decane-7,9-dione dihydrochloride), chloroethylclonidine 2HCl, cloni dine HCl, 5-methyl-urapidil (Research Biochemicals), desipramine HCl, (−)-epinephrine bitartrate, methoxamine HCl, (−)-norepinephrine bitartrate, oxymetazoline HCl, L-phenylephrine HCl, prazosin HCl, WB4101 HCl (2-([2,6- dimethoxyphenoxyethyl] aminomethyl)-1, 4-benzodioxane HCl) (Sigma, St. Louis), St587 (2-(2-chloro-5-trifluoromethyl–phenylimino) -imidazoline) (Boehringer Ingelheim, Ingelheim) and SDZ NVI 085 ((−) - (4a*R*,10a*R*)-3, 4, 4a, 5,10,10a-hexahydro-6-methoxy-4-methyl9-methylthio-2H-naphth[2,3b]-1,4-oxazine hydrogen malonate) (Sandoz, Basel).

All drugs except 5-methyl-urapidil were dissolved in distilled water and 5-methyl-urapidil was dissolved in diluted aqueous acid. These solutions were diluted to the final concentrations with artificial cerebrospinal fluid.

3. Results

3.1. Agonist potencies

The potencies of α_1-adrenoceptor agonists were compared by making extracellular recordings from spinal cord

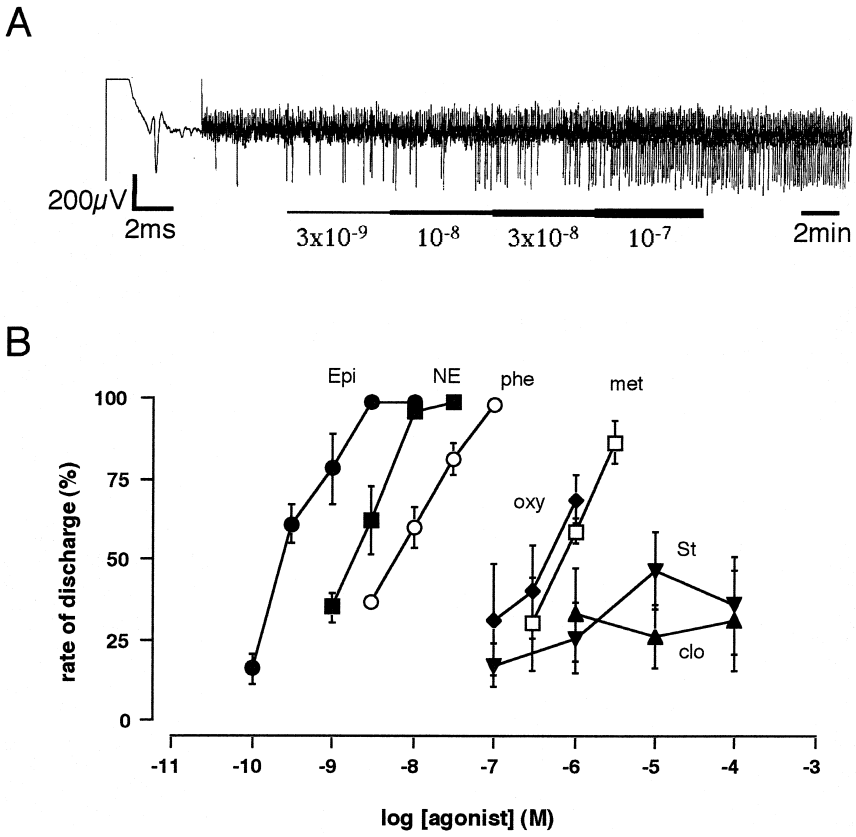

Fig. 1. (A) A typical response of a single cell recorded in the lumbar ventral horn. The response after omission of the artifact was recorded. The bar indicates the phenylephrine concentration. (B) Concentration–response relationships for α_1-adrenoceptor agonists. Each point represents the means ± S.E.M. of 4–5 experiments. Ordinate: the rate of discharge per minute. Abscissa: logarithm of agonist concentration. Abbreviations: Epi, epinephrine; NE, norepinephrine; phe, phenylephrine; met, methoxamine; oxy, oxymetazoline; St, St587; clo, clonidine.

slices isolated from adult rats. A representative trace of the cell discharge induced by phenylephrine is shown in Fig. 1A. The cell discharge was dose dependently facilitated by phenylephrine.

As shown in Fig. 1B, catecholamines (epinephrine, norepinephrine), phenylethylamines (phenylephrine, methoxamine) and imidazolines (clonidine, oxymetazoline, St587) stimulated motoneuron activity. Catecholamines and phenylethylamines gave rise to maximal responses whereas imidazolines gave rise to submaximal responses. Oxymetazoline was the most potent of the imidazolines, but even 10^{-5} M oxymetazoline gave rise to a response of only

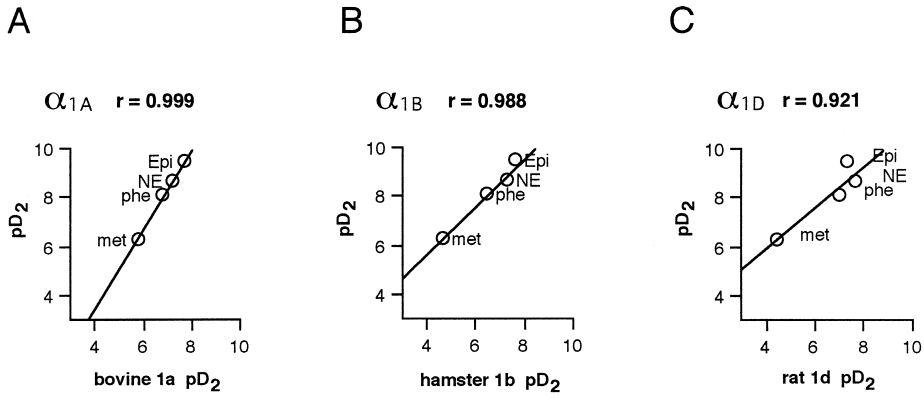

Fig. 2. Comparison of pD_2 values for α_1-adrenoceptor agonists between rat spinal motoneurons (data from our experiments) and cloned α_1-adrenoceptor subtypes (data from Minneman et al., 1994). Ordinates: pD_2 values from our experiments (data from Fig. 1). Abscissae: pD_2 values of cloned α_{1a}- (A), α_{1b}- (B) and α_{1d}- (C) adrenoceptors. Labels next to the open circles represent the following catecholamines or phenylethylamines: Epi, epinephrine; NE, norepinephrine; phe, phenylephrine; met, methoxamine.

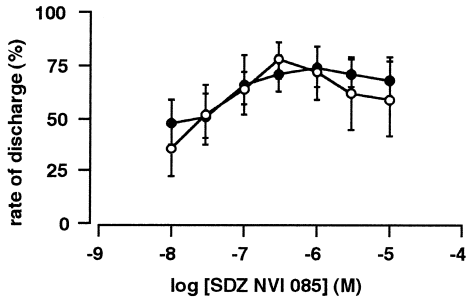

Fig. 3. Effects of the α_{1A}-adrenoceptor agonist SDZ NVI 085 on rat spinal cord motoneurons. Ordinate: the rate of discharge per minute. Abscissa: log of the SDZ NVI 085 concentration. Open circle, SDZ NVI 085 dose–response; closed circle, SDZ NVI 085 dose–response in slices pretreated with 100 μM chloroethylclonidine at 33°C for 30 min followed by a 30-min washout. Data are the means ± S.E.M. of four experiments.

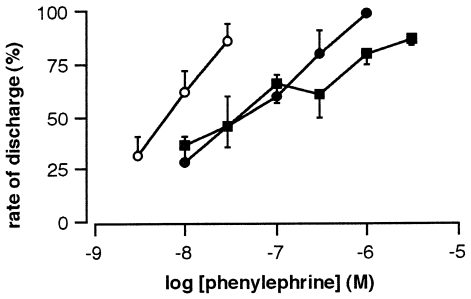

Fig. 4. Effects of chloroethylclonidine treatment on the facilitatory action of phenylephrine. Spinal cord slices were treated at 33°C for 30 min with 0 (open circle), 100 (closed circle) or 300 (closed square) μM chloroethylclonidine followed by a 30 min washout. Data are the means ± S.E.M. of 4–7 experiments.

60% (data not shown). Of the catecholamines, epinephrine was about 6.5 times more higher potent than norepinephrine.

To estimate the potencies of the agonists precisely, the pD_2 values were calculated and the relationship between the potencies of the agonists in this study (ordinates) and their potencies in activating cloned α_1-adrenoceptor subtypes (abscissae) was assessed (Fig. 2). The data for the cloned α_1-adrenoceptor subtypes were taken from a published report (Minneman et al., 1994). Note that cloned α_1-adrenoceptor subtypes are represented by lower case letters. Imidazolines have little or no intrinsic activity at α_{1B}- or α_{1D}-adrenoceptors (Minneman et al., 1994), so the pD_2 values obtained from our studies and from the literature were compared for catecholamines and phenylethylamines but not for imidazolines. The linear correlations (r value) between these two parameters were strongest for the α_{1A}-adrenoceptor and weakest for the α_{1D}-adrenoceptor.

3.2. Selective α_{1A}-adrenoceptor agonist

The α_{1A}-adrenoceptor agonist SDZ NVI 085 facilitated motoneuron activity and behaved as a partial agonist with a maximal response of about 70% (Fig. 3).

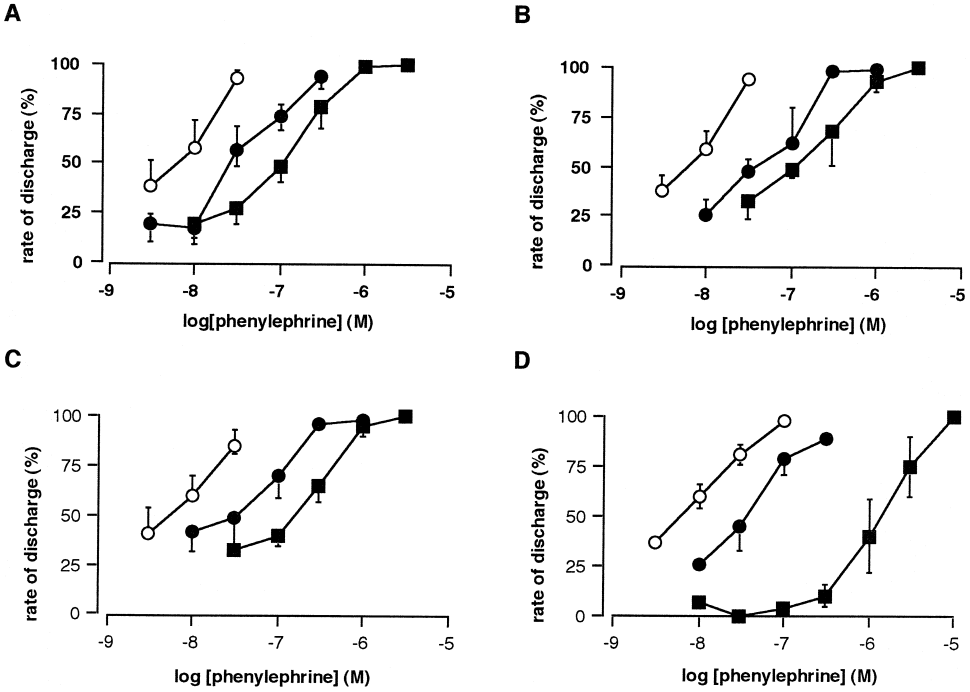

Fig. 5. Effects of competitive α_1-adrenoceptor antagonists on the facilitatory action of phenylephrine. Ordinates: the rate of discharge per minute. Abscissae: log of the phenylephrine concentration. (A) 5-methyl-Urapidil (open circle, control; closed circle, 5-methyl–urapidil 3×10^{-9} M; closed square, 5-methyl-urapidil 10^{-8} M). (B) WB4101 (open circle, control; closed circle, WB4101 10^{-8} M; closed square, WB4101 10^{-7} M). (C) BMY7378 (open circle, control; closed circle, BMY7378 10^{-7} M; closed square, BMY7378 10^{-6} M). (D) Prazosin (open circle, control (the trace of the control is the same data as in Fig. 1); closed circle, prazosin 10^{-9} M; closed square, prazosin 10^{-8} M). Data are the means ± S.E.M. of four experiments.

Table 1
Comparison between estimated antagonist pA_2 values obtained for spinal motoneurons and published antagonist pK_i values for cloned α_1-adrenoceptor subtypes

Drugs	pA_2	Cloned receptor binding (pK_i)		
		1a	1b	1d
Prazosin	10.2	9.4 ± 0.2	9.8 ± 0.2	9.5 ± 0.2
WB4101	8.7	9.5 ± 0.2	8.0 ± 0.2	9.0 ± 0.2
5-methyl-Urapidil	9.2	9.0 ± 0.2	7.0 ± 0.3	7.5 ± 0.3
BMY7378	7.5	6.2	6.3	8.2

Schild plots were made from the dose–response curves shown in Fig. 5 to obtain the pA_2 values. Data except those for BMY7378 are the means ± S.E.M. of five values from the literature. Data for BMY7378 are the means of two values. References: Faure et al., 1994; Goetz et al., 1994, 1995; Kenny et al., 1994; Testa et al., 1995.

3.3. Chloroethylclonidine treatment

Perez (1991) reported that α_{1B}- and α_{1D}-adrenoceptors are inactivated by chloroethylclonidine. Treatment of rat spinal cord slices with 100 μM chloroethylclonidine caused a rightward shift of the phenylephrine dose–response curve, almost without affecting the maximum response (Fig. 4). Treatment with a high concentration of chloroethylclonidine (300 μM) gave almost the same result as 100 μM chloroethylclonidine. Therefore, the lower concentration of chloroethylclonidine (100 μM) seems to be sufficient to alkylate this tissue. In the presence of chloroethylclonidine (100 μM, 30 min), the response induced by the α_{1A}-adrenoceptor agonist SDZ NVI 085 was not inhibited (Fig. 3).

3.4. Antagonist potencies

The effects of α_1-adrenoceptor antagonists were examined. Prazosin, 5-methyl-urapidil, WB4101 and BMY7378 each concentration dependently inhibited the facilitation of motoneuron activity induced by phenylephrine (Fig. 5). BMY7378 was about 10-fold weaker than the other antagonists. To compare the potencies of the antagonists precisely, the pA_2 values for the antagonists were calculated and are shown in Table 1. The reported pK_i values for individual antagonists were taken from the literature (Faure et al., 1994; Goetz et al., 1994, 1995; Kenny et al., 1994; Testa et al., 1995) and averaged. These pK_i values suggest the following characteristics (Table 1, cloned pK_i values. Note that cloned α_1-adrenoceptor subtypes are represented by lower case letters). BMY7378 and 5-methyl-urapidil have strong affinity for α_{1A}- and α_{1D}-adrenoceptors, respectively. Prazosin has strong affinity for all subtypes and WB4101 has weak affinity for α_{1B}-adrenoceptors. In this experiment, the order of potency of the antagonists was similar to that of their α_{1A}-adrenoceptor binding affinity given in the literature. Note that 5-methyl-urapidil has higher affinity for α_{1A}-adrenoceptors than does BMY7378, and conversely, BMY7378 has higher affinity for α_{1D}-adrenoceptors than 5-methyl-urapidil according to the published reports. In our experiment, 5-methyl-urapidil inhibited the excitation of motoneurons with greater potency than did BMY7378. These results indicate that the α_1-adrenoceptors of rat spinal cord motoneurons are predominantly 5-methyl-urapidil sensitive.

4. Discussion

We previously reported the subtypes of α_1-adrenoceptor present in rat spinal cord (Wada et al., 1996). In the rat ventral horn, α_{1A}-adrenoceptors, and not α_{1B}-adrenoceptors, are the main subtype and α_{1D}-adrenoceptors are sparsely distributed. Spinal motoneurons are mainly located in the ventral horn, therefore the predominance of α_{1A}-adrenoceptors in that region suggests that the α_{1A}-adrenoceptor plays an important role in facilitating spinal motoneuron activity. In the present study, we assessed the possibility that the α_{1A}-adrenoceptor exerts a facilitatory effect on rat spinal cord motoneurons, using an electrophysiological procedure.

In our previous studies, it has been shown that the α_2-adrenoceptor agonist clonidine has a facilitatory action in rat spinal cord, and that this effect is blocked by low concentrations of prazosin. In addition, only in the presence of prazosin did the α_2-adrenoceptor-mediated suppressive action of clonidine appear (Hirayama et al., 1988a: Tanabe et al., 1990). Kehne et al. (1985) reported that clonidine facilitates the flexor reflex through stimulation of spinal α_1-adrenoceptors. Therefore, in the spinal motoneuron, clonidine predominantly mediates the facilitatory effect via α_1-adrenoceptors, and the α_2-adrenoceptor-mediated suppressive action is usually masked by this α_1-adrenoceptor-mediated facilitatory action. The other imidazolines, like clonidine, showed facilitatory activity in this study (Fig. 1). Imidazolines are selective for the α_{1A}-adorenoceptor subtype and have little or no intrinsic activity at the other two subtypes (Minneman et al., 1994). Oxymetazoline, which is one of the imidazolines, is a weak agonist of α_{1a}-adrenoceptors and has no intrinsic activity at the other subtypes (Horie et al., 1995). Clonidine induces contraction of rabbit thoracic aorta via the α_{1A}-adrenoceptor (Satoh et al., 1992). Therefore, the facilitative effects of imidazolines on spinal cord motoneurons are induced by α_{1A}-adrenoceptor activation. Phenylethylamines, and especially methoxamine, show small variations in their activation of the three α_1-adrenoceptor subtypes, but they have relatively low potencies at the α_{1B}-adrenoceptor (Minneman et al., 1994). Methoxamine has no contractile activity up to 10^{-3} M in guinea-pig spleen which is thought to mainly contain α_{1B}-adrenoceptors (Eltze and Boer, 1992). In the present study, methoxamine had a facilitatory action, suggesting that α_{1B}-adrenoceptors are not the main subtype in rat spinal motoneurons.

The α_{1A}-adrenoceptor agonist SDZ NVI 085 also had a facilitatory effect on rat spinal motoneurons (Fig. 3). SDZ NVI 085 has been reported to display high affinity for α_{1A}-adrenoceptors but low affinity for α_{1B}-adrenoceptors (Renaud et al., 1991). Accordingly, at low concentrations, SDZ NVI 085 elicits smooth muscle contraction in tissues with α_{1A}-adrenoceptor supply, but not in rat aorta (Eltze and Boer, 1992) where the contractions are suggested to be induced via α_{1D}-adrenoceptors (Testa et al., 1995). In guinea-pig spleen, which is thought to contain α_{1B}-adrenoceptors (Eltze, 1994), SDZ NVI 085 does not evoke splenic contraction at concentrations lower than 10^{-4} M. Therefore, we think that, in the present study, SDZ NVI 085 made the excitation of motoneurons easier via a α_{1A}-adrenoceptor mechanism. Certainly, the effect of SDZ NVI 085 was not inhibited by treating the slices with the α_{1B}- and α_{1D}-adrenoceptor alkylating agent chloroethylclonidine (100 μM, 30 min), so SDZ NVI 085 behaved as an α_{1A}-adrenoceptor agonist. The data obtained for the imidazolines, methoxamine and SDZ NVI 085 suggest that α_{1A}-adrenoceptors are predominantly involved with the facilitation of spinal motoneuron activity.

The linear correlation between the pD_2 values for the motoneurons and those for the cloned α_1-adrenoceptor subtypes was strongest for the α_{1A}-adrenoceptor (Fig. 2). These data also indicate that the potencies of the agonists on the motoneurons were similar to their potencies for α_{1A}-adrenoceptor activation and that facilitation of motoneuron activity is mediated mainly via α_{1A}-adrenoceptors.

In the experiments with antagonists, α_1-adrenoceptors were activated by phenylephrine. Norepinephrine may be the best agonist for activating all α_1-adrenoceptor subtypes, but it may also activate α_2-adrenoceptors. Hence, phenylephrine was used. Though phenylephrine is known to be less potent at α_{1B}-adrenoceptors, phenylephrine was a full agonist in this study (Fig. 1). Phenylephrine was previously used to isolate the α_1-adrenoceptor component of contractions in rat spleen (Burt et al., 1995). Thus, the use of phenylephrine in this study seems appropriate. The pA_2 values of the antagonists were estimated during phenylephrine-induced facilitation of motoneuron activity. The order of potency of the antagonists was similar to that for their α_{1A}-adrenoceptor binding site affinity (Table 1). Therefore, in the antagonist experiment, as in the agonist experiment, α_{1A}-adrenoceptors were shown to be predominant in the facilitation of rat spinal motoneuron activity, although there were some differences between the pA_2 and pK_i values for several antagonists. This may have been because some of the α_1-adrenoceptor antagonists have high affinity for 5-hydroxytryptamine receptors (Millan et al., 1994; Goetz et al., 1995) and 5-hydroxytryptamine has some effect on the spinal cord (Ono et al., 1991; Yamazaki et al., 1992a,b).

Are α_1-adrenoceptor subtypes other than α_{1A}-adrenoceptors involved in this facilitation of motoneuron activity? First, we will discuss α_{1D}-adrenoceptors. Few α_{1D}-adrenoceptors have been detected in the rat spinal ventral horn (Wada et al., 1996). Our pA_2 values for BMY7378 were lower than the published pK_i values for the α_{1D}-adrenoceptor (Table 1). The linear correlation between the pD_2 values for the spinal motoneurons and the cloned α_1-adrenoceptor subtypes was weakest for the α_{1D}-adrenoceptor (Fig. 2). These facts suggest that the α_{1D}-adrenoceptors are less important than the other subtypes in facilitating motoneuron activity of rat spinal cord. Moreover, the relative potencies of norepinephrine and epinephrine may indicate the involvement of the α_1-adrenoceptor subtypes. The order of pD_2 values of epinephrine and norepinephrine was epinephrine > norepinephrine at α_{1A}- and α_{1B}-adrenoceptors, but norepinephrine > epinephrine at α_{1D}-adrenoceptors (Minneman et al., 1994). Epinephrine has a higher potency than norepinephrine in rat vas deferens and guinea-pig spleen (α_{1A}- and α_{1B}-adrenoceptors are predominant, respectively), but the order is reversed in rat aorta (α_{1D}-adrenoceptors are predominant) (Eltze and Boer, 1992; Eltze, 1994). In our experiment, norepinephrine was about 6.5-fold less potent than epinephrine in spinal cord slices, suggesting functionally less participation of α_{1D}-adrenoceptors (Fig. 1).

Next, we consider the α_{1B}-adrenoceptor. In the present study, a small proportion of spinal motoneuron activity was inactivated by an α_{1B}- and α_{1D}-adrenoceptor alkylating agent, chloroethylclonidine, when spinal motoneurons were activated by phenylephrine (Fig. 4). The α_{1A}-adrenoceptor agonist, SDZ NVI 085, behaved as a partial agonist (Fig. 3). It has been reported that SDZ NVI 085 behaves as a full α_1-adrenoceptor agonist in rabbit ear artery (Nozulak et al., 1992), in which mRNA for α_{1B}-adrenoceptor is not detected (Schwinn et al., 1991). Therefore, the small proportion of motoneuron activity which was inactivated by chloroethylclonidine and was not activated by SDZ NVI 085 may be mediated by α_{1B}-adrenoceptors. The α_{1B}-adrenoceptor is not the major receptor but does play a functional role in rat spinal motoneuron activity.

In one of the few reports that discuss the functional roles of α_1-adrenoceptor subtypes in rat spinal cord, Bervoets and Millan (1994) suggest that spinal α_{1A}-adrenoceptors mediate the spontaneous tail flicks induced by 8-OH-DPAT. In regard to the α_{1A}-adrenoceptor being predominant, our results are similar to theirs although their experiment was in vivo and was not limited to the ventral horn or to motoneurons. In another report, in which the functional roles were not discussed, Wilson and Minneman (1989) showed that in cervical spinal cord, 42% of the α_1-adrenoceptors of the spinal cord are inactivated by chloroethylclonidine, and that the α_{1A}-adrenoceptor is the predominant subtype in the rat cervical spinal cord. Our results, obtained from electrophysiological experiments, are consistent with their results in spite of the use of different regions of the spinal cord.

We could not verify whether the motoneurons studied were innervating flexor or extensor muscles, since we used isolated spinal cord slice preparations. It is known that 5-HTP mainly increases extensor activity, while L-DOPA or clonidine may increase flexor as well as extensor activity (Conway et al., 1988; Hounsgaard et al., 1988). Therefore, it is possible that the activity of flexor and extensor motoneurons can be differentially facilitated by the α_1-adrenoceptor subtypes. However, clonidine, which is a preferential α_{1A}-adrenoceptor agonist, facilitated the activity of both flexors and extensors (Conway et al., 1988) so that α_{1A}-adrenoceptors may be present in both neurons. These facts may mean that both flexor and extensor motoneurons have the same characteristic α_1-adrenoceptor subtypes. However, further experiments are needed to answer this question.

In conclusion, our results suggest that α_{1D}-adrenoceptors, which are expressed at low levels in the ventral horn, barely participate in the facilitation of motoneuron activity, and that the α_{1A}-adrenoceptor, and not the α_{1B}-adrenoceptor, is the main subtype involved in the facilitation of rat spinal cord motoneuron activity.

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