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Original Article

Epinephrine, phenylephrine, and methoxamine induce infiltrative anesthesia via α1-adrenoceptors in rats

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Aim: To assess whether epinephrine, phenylephrine, and methoxamine act via certain subtypes of adrenoceptors to exert their local anesthetic activity.
Methods: We investigated cutaneous anesthesia from adrenoceptor agonists and/or antagonists in conscious, unanesthetized Sprague-Dawley male rats (weight 200−250 g). Cutaneous anesthesia was evidenced by a block of the cutaneous trunci muscle reflex, which is characterized by reflex movement of the skin over the back produced by twitches of lateral thoracispinal muscles in response to local dorsal cutaneous noxious pinprick.
Results: Local infiltration of epinephrine, *L*-phenylephrine, or methoxamine alone induces cutaneous anesthesia in rats in a dose-dependent way. Epinephrine is found to be 19 and 29 times more potent than those of methoxamine and *L*-phenylephrine, respectively. The cutaneous anesthesia induced by epinephrine, phenylephrine, or methoxamine can be significantly reduced by α_1-adrenoceptor antagonists (*eg*, prazosin), α1, α2-adrenoceptor antagonist, α_{1A}-adrenoceptor antagonist (*eg*, 5-methylurapdil), α_{1B}-adrenoceptor antagonist (eg, chloroethylclonidine), or α_{1D}-adrenoceptor antagonist (*eg*, BMY7873).
Conclusion: Our results indicate that epinephrine, phenylephrine and methoxamine all act mainly via mixed subtypes of α_1-adrenoceptors to induce cutaneous anesthesia in the rat.

Keywords: anesthesia; epinephrine; vasoconstriction; phenylephrine; methoxamine

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Introduction

Anesthesiologists often add epinephrine to local anesthetic preparations during peripheral nerve block procedures[1, 2]. It is generally believed that epinephrine mediates this prolongation of local anesthetic action by its vasconstrictive action[3]. First, epinephrine may reduce the plasma concentration of local anesthetic and thus minimize the possibility of systemic toxicity[4], and second, epinephrine potentiates peripheral nerve block[2, 5, 6]. Epinephrine may stimulate α-adrenoceptors receptors on the neural vasculature[7], which leads to contraction of the vascular smooth muscle[8, 9], and reduction of both local blood flow and clearance of local anesthetic from the nerve.

It has been documented that norepinephrine, phenylephrine, or methoxamine injected intradermally may induce thermal hyperalgesia in humans[10], and that epinephrine can produce mechanical hyperalgesia in rats[11]. In contrast, recent findings[12, 13] observe that the epinephrine itself induces an unexpected, transient, partial block of the cutaneous trunci muscle reflex, which is characterized by reflex movement of the skin over the back produced by twitches of lateral thoracispinal muscles in response to local dorsal cutaneous noxious pinprick. This raises the possibility that epinephrine, phenylephrine or methoxamine may possess local anesthetic activity in its own right.

To deal with the question, the authors undertook this study to determine a dose-response curve for epinephrine, phenylephrine, or methoxamine on infiltrative anesthesia on conscious, unanesthetized rats. In order to determine whether epinephrine, phenylephrine, and methoxamine act via certain subtypes of α1-adrenoceptors to exert their cutaneous analgesia, local infiltration of different α-adrenoceptor antagonists was made 5 min before injection of epinephrine, phenylephrine, or methoxamine. In addition, we injected the nitric oxide donors (such as nitroglycerin, niroprusside or nifedipine) 5 min before injection of epinephrine, phenylephrine, or methoxamine during cutaneous anesthesia testing to ascertain whether vasoconstriction affected local anesthesia.

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Materials and methods

Animals

We investigated local anesthesia from adrenergic agonists and/or antagonist in conscious, unanesthetized Sprague-Dawley male rats (weight 200−250 g). All experiments were performed by using protocols approved by the Chi Mei Medical Center Committee on Animals in accordance with policies of the International Association for the Study of Pain. All rats were housed in groups of three to four for at least one week in a climate-controlled room maintained at 21 °C with approximately 50% humidity. A 12-h light/dark cycle was settled with food and water available *ad libitum* till the time of investigation.

The experiments were done on handled rats (daily, over 7 days) familiarized with the behavioral experimenter, the laboratorial environment, and the specific procedures of testing. Such familiarization minimized the contamination of animals from stress during experiments and improved experimental performance[14]. The hairs of the dorsal surface of the thoracolumbar region (6×6 cm^2) of rats were mechanically clipped the day before experiments and this small degree of local irritation by clipping disappeared overnight. Six to eight rats in each group were assigned for different treatments.

Evaluation of local anesthesia

Local anesthesia from different adrenoceptor agonists and antagonists were evaluated according to the method reported previously[14, 15]. In brief, drugs were administered via a 30-gauge needle at a volume of 0.6 mL subcutaneously at a 30° angle into the dorsal surface of the thoracolumbar region. The injections caused a circular raise of the skin, a wheel, approximately two centimeter in diameter that was then marked with ink within 1 min. The effect of the local anesthesia was evaluated using the cutaneous trunci muscle reflex, which was characterized by reflex movement of the skin over the back produced by twitches of the lateral thoracispinal muscles in response to local dorsal subcutaneous stimulation. A von Frey filament (No 15), to which the cut end of an 18-gauge needle was affixed, was used to produce the standardized nociceptive stimulus (19 g). We performed six different pinpricks inside the wheal with a frequency of 0.5−1.0 Hz after observing an animal's normal reaction to pinpricks applied outside the wheal and on the contralateral side and scoring the number to which the rat failed to react. The investigation was applied every 5 min for the first 30 min and then every 10–15 min to 2.5 h until the subcutaneous reflex completely recovered from the blockage. The back was subdivided into four areas on both sides, and each rat was injected 2 times, separated by a washout period of 3 days. For consistency, one experienced investigator, who was unaware of the drugs being injected, was responsible to evaluate cutaneous analgesia effects.

Drugs

All the drugs were freshly prepared. The following drugs were used: α1-agonists (*L*-phenylephrine HCl; Sigma, methoxamine HCl; Sigma), α_2-agonist (clonidine; Sigma, dexmedetomidine; Abbott), β_1-agonist (dobutamine; Astra Zeneca), β_2-agonist (terbutaline; Sigma), $\beta_1\beta_2$-agonist (isoproterenol; Sigma), $\alpha_1\alpha_2\beta_1\beta_2$ agonist (epinephrine; Sigma), α_1-antagonist (prazosin; Sigma), $\alpha_1\alpha_2$-antagonist (phentolamine; Sigma), α_{1A} antagonist (5-methylurapdil; Sigma), α_{1B} antagonist (chloroethylclonidine; Sigma), α_{1D} antagonist (BMY7378; Sigma), Ca^{2+} and α_1A antagonist (nifedipine; Sigma) and NO donor vasodilator (sodium nitroprusside; Mulgrave VIC, nitroglycerine; Nippon Kayaku, nifedipine; Sigma). All compounds were dissolved in isotonic saline except prazosin, which was firstly dissolved in polyethylene glycol followed by dilution with isotonic saline.

Experimental procedures

The potencies of drugs on cutaneous analgesia was evaluated. The fitting of dose-response curves of each drug was constructed from percent maximum possible effect (%MPE). In the antagonism studies, we injected the antagonist (at a volume of 0.3 mL) firstly and then the agonist was administered (at a volume of 0.3 mL) 5 min later. After subcutaneous injection (n=6 rats for each dose of each drug), the %MPE of doses of drugs were obtained. The dose-response curves of drugs were then constructed using the %MPE and fitted with a computer-derived SASNLIN analysis (version 9.1, SAS Institute, NC). The values of 50% effective doses (ED50s) of drugs, which were defined as the doses of drugs that caused a 50% blockage of cutaneous trunci muscle reflex, were obtained[16].

Statistical analysis

Values were presented as mean±SEM. The differences in ED_{50s} among drugs were evaluated by a one-way analysis of variance (ANOVA), followed by the pariwise Tukey's honest significance difference (HSD) test. A statistical software, SPSS for windows (version 10, 0.7), was used. A P value <0.05 was considered statistically significant.

Results

Epinephrine, phenylephrine, or methoxamine induces infiltrative anesthesia

It can be seen from both Figire 1 and Table 1 that epinephrine, phenylephrine, methoxamine, and norepinephrine induce infiltrative anesthesia in a dose-dependent way in rats.

Table 1. Maximum possible effect of drugs on subcutaneous antinociception in rats.

Drugs	μmol·L^{-1}/mL	%MPE
Clonidine	1 : 1×10^5	0
Dexmedetomidine	1 : 1×10^5	0
Dobutamine	1 : 1×10^4	0
Terbutamine	1 : 1×10^4	0
Isoproterenol	1 : 1×10^4	0
Norepinephrine	1 : 1×10^4	81±12
Norepinephrine	1 : 1×10^5	57±10

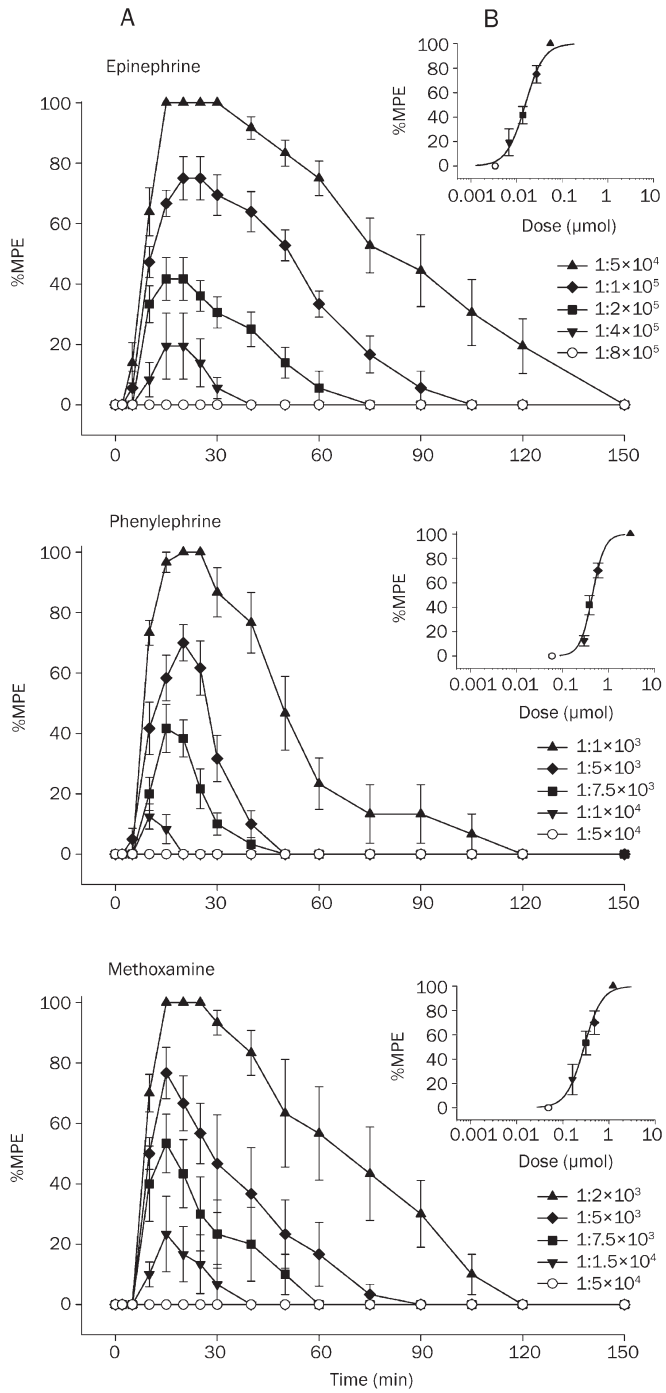

Figure 1. (A) Time courses of inhibition of cutaneous trunci muscle reflex by epinephrine, phenylephrine, and methoxamine after subcutaneous injections of drugs in rats (n=6 rats for each drug). Values are mean±SEM. The injected volume was 0.6 mL. Neurological evaluations were done before, and 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min after drug injection. (B) The dose-response curves of epinephrine, phenylephrine, and methoxamine on cutaneous analgesia in rats (n=6 rats at each testing point). Values are mean±SEM and were fitted with the SASNLIN analysis.

Epinephrine is found to be 19 and 29 times more potent than those of methoxamine and *L*-phenylephrine, respectively. In addition, the relative potency was found to be epinephrine > lidocaine. On the other hand, clonidine, dexmedetomidine, dobutamine, and terbutaline all exhibit no infiltrative anesthesia.

Alpha-adrenoceptor antagonists reduce infiltrative anesthesia induced by epinephrine, phenylephrine, or methoxamine

In order to determine the effects of antagonism of α_1,α_2-adrenoceptors on the infiltrative anesthesia induced by epinephrine, phenylephrine, or methoxamine, prazosin or phentolamine was administered 5 min before injection of these adrenoceptor agonists. It can be seen from both Figure 2 and Figure 3 that infiltrative anesthesia induced by epinephrine, phenylephrine, or methoxamine can be significantly reduced by prazosin (Figure 2) or phentolamine (Figure 3).

Mixed subtypes of α1-adrenoceptor antagonists reduce infiltrative anesthesia induced by epinephrine, phenylephrine, or methoxamine

It can be seen from Figure 4, Figure 5, and Figure 6 that the infiltrative anesthesia induced by epinephrine, phenylephrine, or methoxamine can be significantly abolished by pretreatment with α_{1A} (5-methylurapdil), α_{1B} (chloroethylclonidine), or α_{1D} (BMY7873) adrenoceptor antagonist 5 min before the injection of epinephrine, phenylephrine, or methoxamine.

Nitric oxide donors attenuate infiltrative anesthesia caused by epinephrine, phenylephrine, or methoxamine

As summarized in Table 2, the infiltrative anesthesia caused by epinephrine, phenylephrine, or methoxamine can be completely abolished by pretreatment with nitroglycerin, nitroprusside, or nifedipine 5 min before the injection of epinephrine, phenylephrine or methoxamine.

Discussion

Both clinical and preclinical studies have indicated that the sympathetic nervous system contributes to pain following nerve injury[17, 18]. It is generally believed that sympathetic-afferent coupling occurs at three distinct sites; at the site of injury, at the sensory terminal, and within dorsal root ganglia. Sympathectomy can relieve the different manifestations of hyperalgesia and allodynia in various nerve injury models to varying degrees[19–21]. In addition, both behavioral and electrophysiological studies suggest that α2-adrenoceptors are primarily mediators of sympathetic-afferent coupling following nerve injury[22–25]. Both α2-[18, 26] and α1-adrenoceptors[24, 27] are related to afferent excitation following nerve injury. Clonidine, an α_2-adrenoceptor agonist commonly used in the treatment of hypertension, has been used to relieve hyperalgesia in some patients with sympathetically maintained pain due to a localized action[28]. The efficacy of local clonidine in sympathetically maintained pain may result from presynaptic inhibition of norepinephrine released from sympathetic nerves as well as actions directly on primary afferent nerve terminals.

Probably, the most striking findings of the present study are

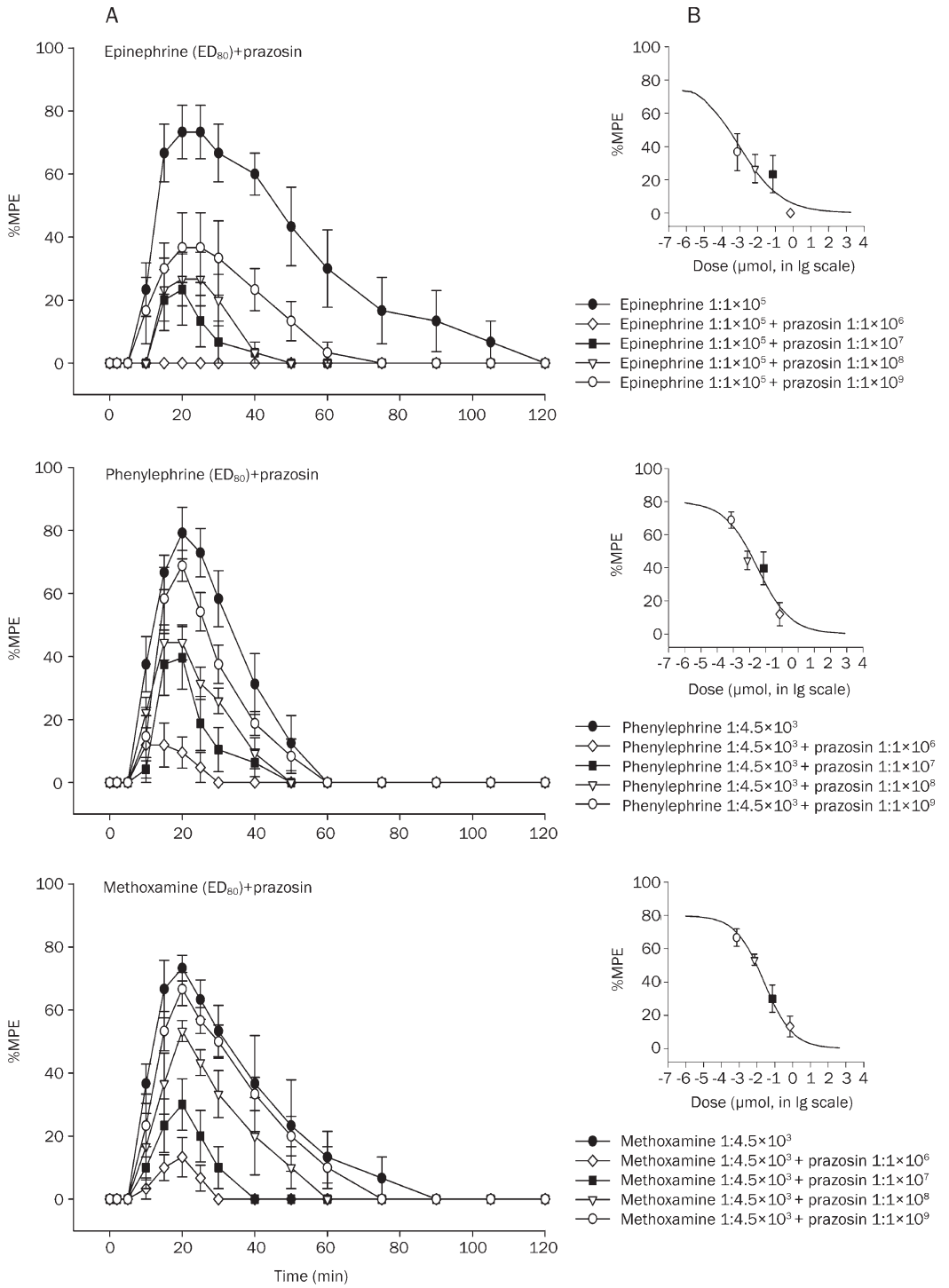

Figure 2. (A) Time courses of inhibition of cutaneous trunci muscle reflex by epinephrine (ED_{80}), phenylephrine (ED_{80}), and methoxamine (ED_{80}) in the presence of prazosine after subcutaneous injections of drugs in rats (*n*=6 rats for each drug). Values are mean±SEM. Prazosine (0.3 mL) was injected 5 min before the injection of epinephrine (0.3 mL), phenylephrine (0.3 mL), and methoxamine (0.3 mL). Neurological evaluations were done before, and 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min after drug injection. (B) The dose-response curves of epinephrine, phenylephrine, and methoxamine in the presence of prazosine on cutaneous analgesia in rats (*n*=6 rats at each testing point). Values are mean±SEM and were fitted with the SASNLIN.

that α_1-adrenoceptor agonists (*eg*, epinephrine, phenylephrine, and methoxamine) but not α_2-adreneceptor agonists (*eg*, clonidine and dexmedetomidine), β_1-adrenoceptor agonist (*eg*, dobutamine), β_2-adrenoceptor agonist (*eg*, terbutaline), or $\beta_1\beta_2$-

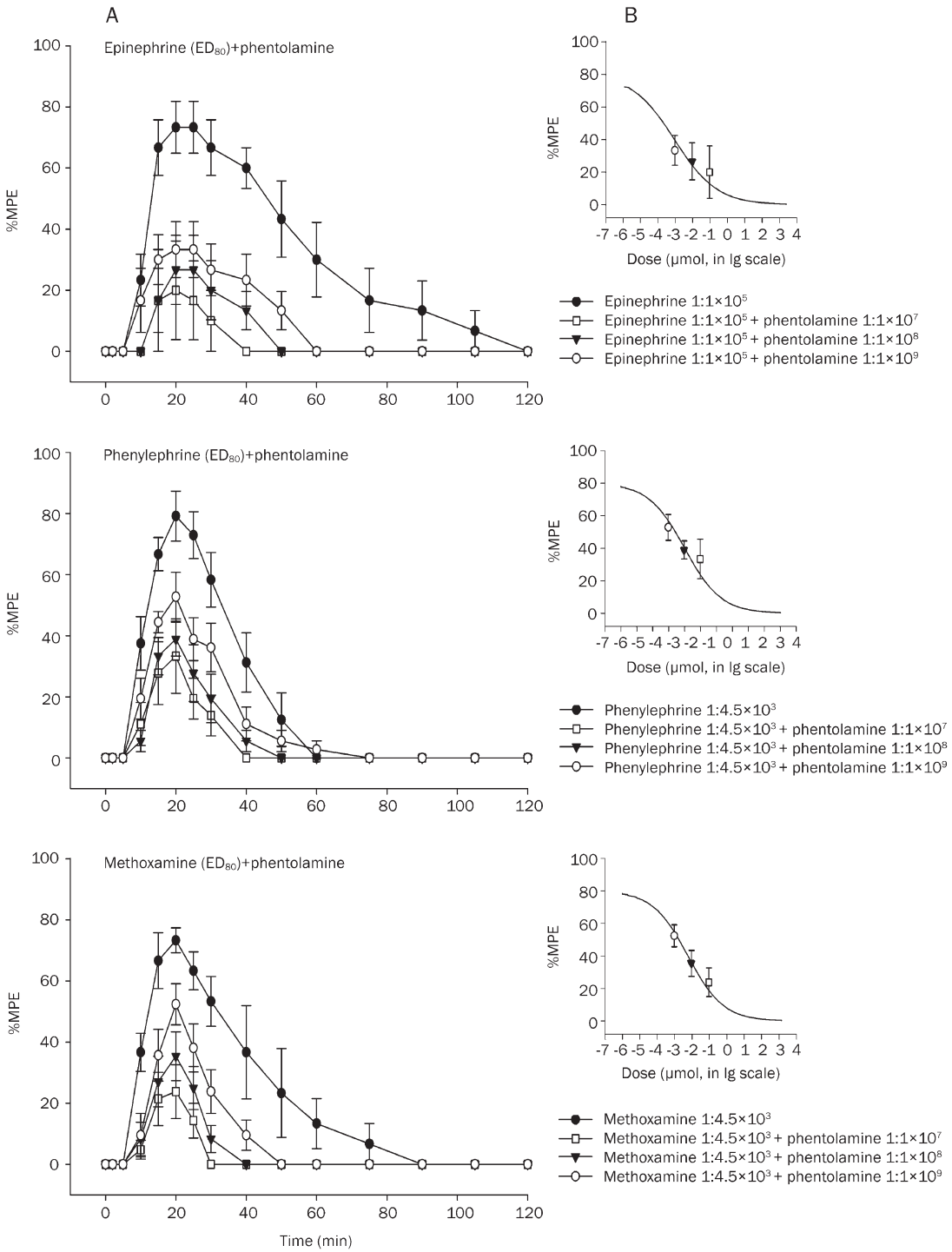

Figure 3. (A) Time courses of inhibition of cutaneous trunci muscle reflex by epinephrine, phenylephrine, and methoxamine in the presence of phentolamine after subcutaneous injections of drugs in rats (n=6 rats for each drug). Values are mean±SEM. Phentolamine (0.3 mL) was injected 5 min before the injection of epinephrine (0.3 mL), phenylephrine (0.3 mL), and methoxamine (0.3 mL). Neurological evaluations were done before, and 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min after drug injection. (B) The dose-response curves of epinephrine, phenylephrine, and methoxamine in the presence of phentolamine on cutaneous analgesia in rats (n=6 rats at each testing point). Values are mean±SEM and were fitted with the SASNLIN.

adrenoceptor agonist (*eg*, isoproterenol) induce infiltrative anesthesia in a dose-related manner after subcutaneous infiltration in the rat. Epinephrine is found to be more potent than that of lidocaine (present results) or bupivacaine[13]. The

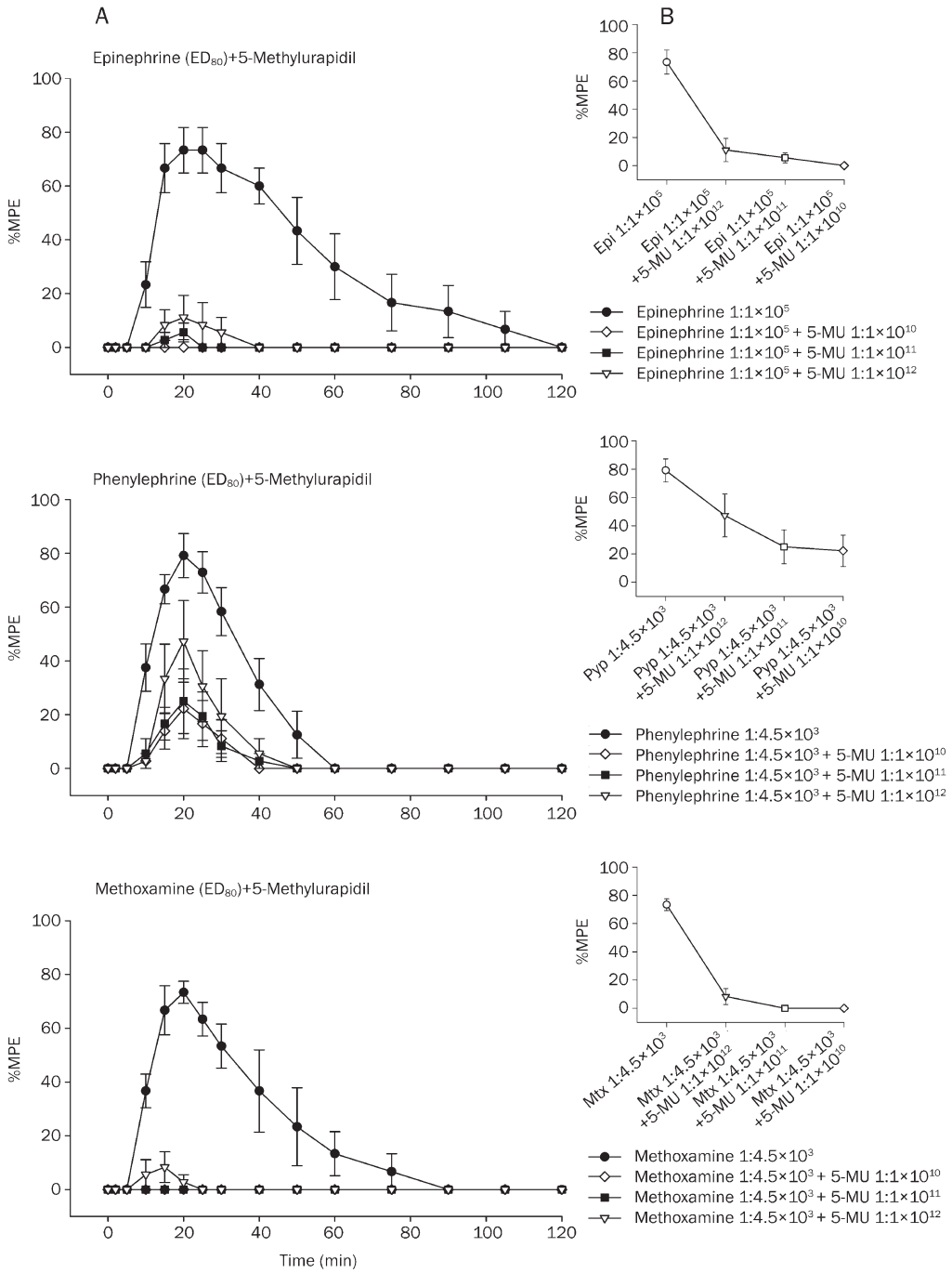

Figure 4. (A) Time courses of inhibition of cutaneous trunci muscle reflex by epinephrine, phenylephrine, and methoxamine in the presence of 5-methylurapidil after subcutaneous injections of drugs in rats (n=6 rats for each drug). Values are mean±SEM. 5-Methylurapidil (0.3 mL) was injected 5 min before the injection of epinephrine (0.3 mL), phenylephrine (0.3 mL), and methoxamine (0.3 mL). Neurological evaluations were done before, and 5, 10, 15, 20, 25, 30, 40 , 50, 60, 75, 90, 105, and 120 min after drug injection. (B) The dose-response curves of epinephrine, phenylephrine, and methoxamine in the presence of 5-methylurapidil on cutaneous analgesia in rats (n=6 rats at each testing point). Values are mean±SEM and were fitted with the SASNLIN.

infiltrative anesthesia induced by epinephrine, phenylephrine, or methoxamine can be significantly reduced by α_1-antagonist (*eg* prazosin), $\alpha_1\alpha_2$-antagonist (*eg*, phentolamine), α_{1A}-adrenoceptor antagonist (*eg*, 5-methylurapidil), α_{1B}-adrenoceptor antagonist (*eg*, chloroethylclonidine), or α_{1D}-adrenoceptor antagonist (*eg*, BMY7873). These results indicate that epinephrine, phenylephrine, or methoxamine can act mainly via mixed subtypes of α_1-adrenoceptors to

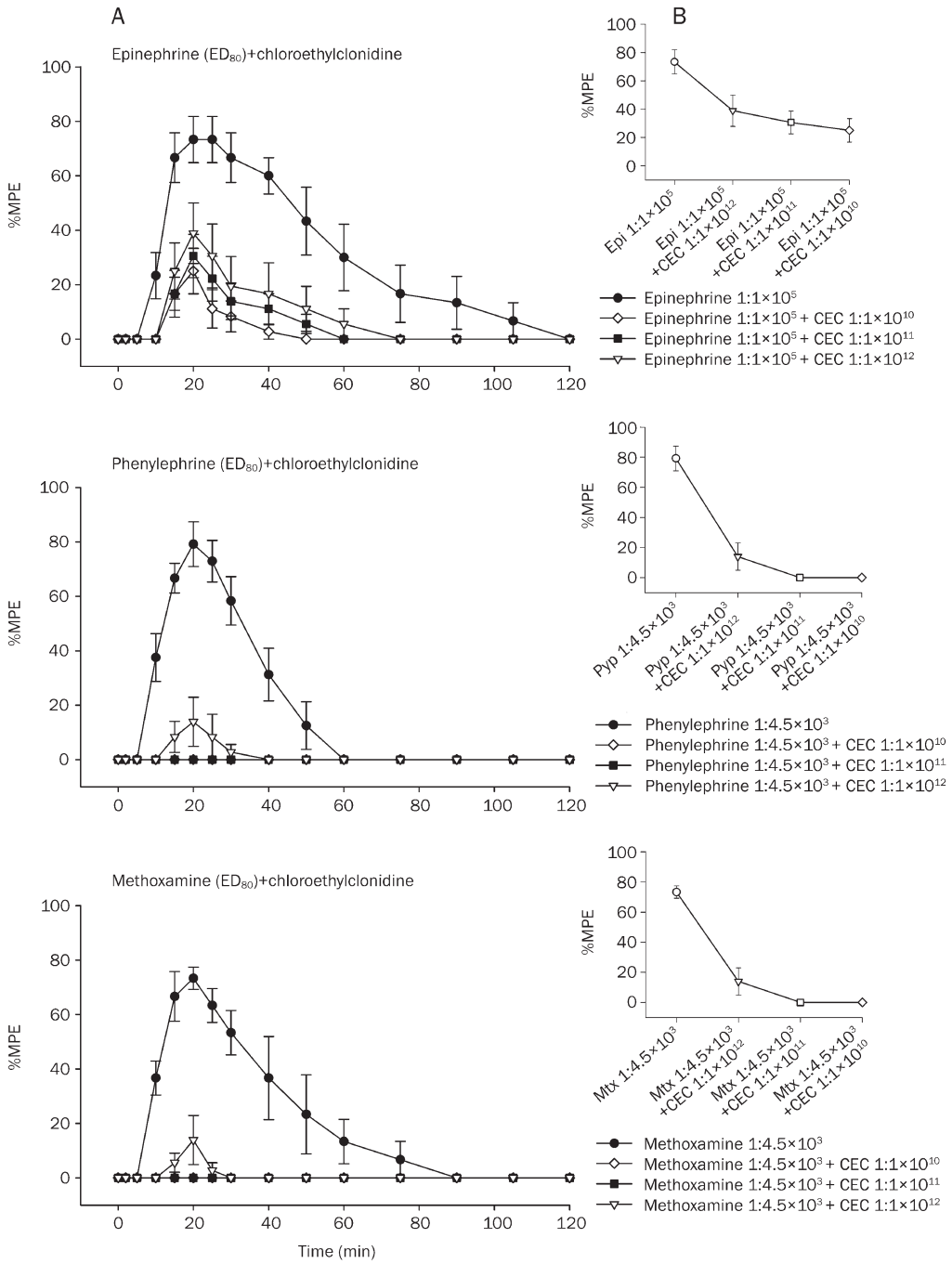

Figure 5. (A) Time courses of inhibition of cutaneous trunci muscle reflex by epinephrine, phenylephrine, and methoxamine in the presence of chloroethylclonidine (CEC) after subcutaneous injections of drugs in rats (*n*=6 rats for each drug). Values are mean±SEM. CEC (0.3 mL) was injected 5 min before the injection of epinephrine (0.3 mL), phenylephrine (0.3 mL), and methoxamine (0.3 mL). Neurological evaluations were done before, and 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min after drug injection (B). The dose-response curves of epinephrine, phenylephrine, and methoxamine in the presence of CEC on cutaneous analgesia in rats (*n*=6 rats at each testing point). Values are mean±SEM and were fitted with the SASNLIN.

induce cutaneous analgesia in the rat. In fact, the contention is not consistent with a more recent report showing that α-2 adrenoceptor agonists enhance the local anesthetic action of lidocaine, and suggest that dexmedetomidine (which has more than eight times the affinity for α-2 adrenoceptors of clonidine) acts via α-2A adrenoceptors in guinea pigs[29]. It should be noted that they showed that all α-2 adrenoceptor agonists enhanced the degree of local anesthesia of lidocaine in a dose-dependent manner but did not demonstrate the effects of clonidine or dexmedetomidine itself on local anesthesia. In

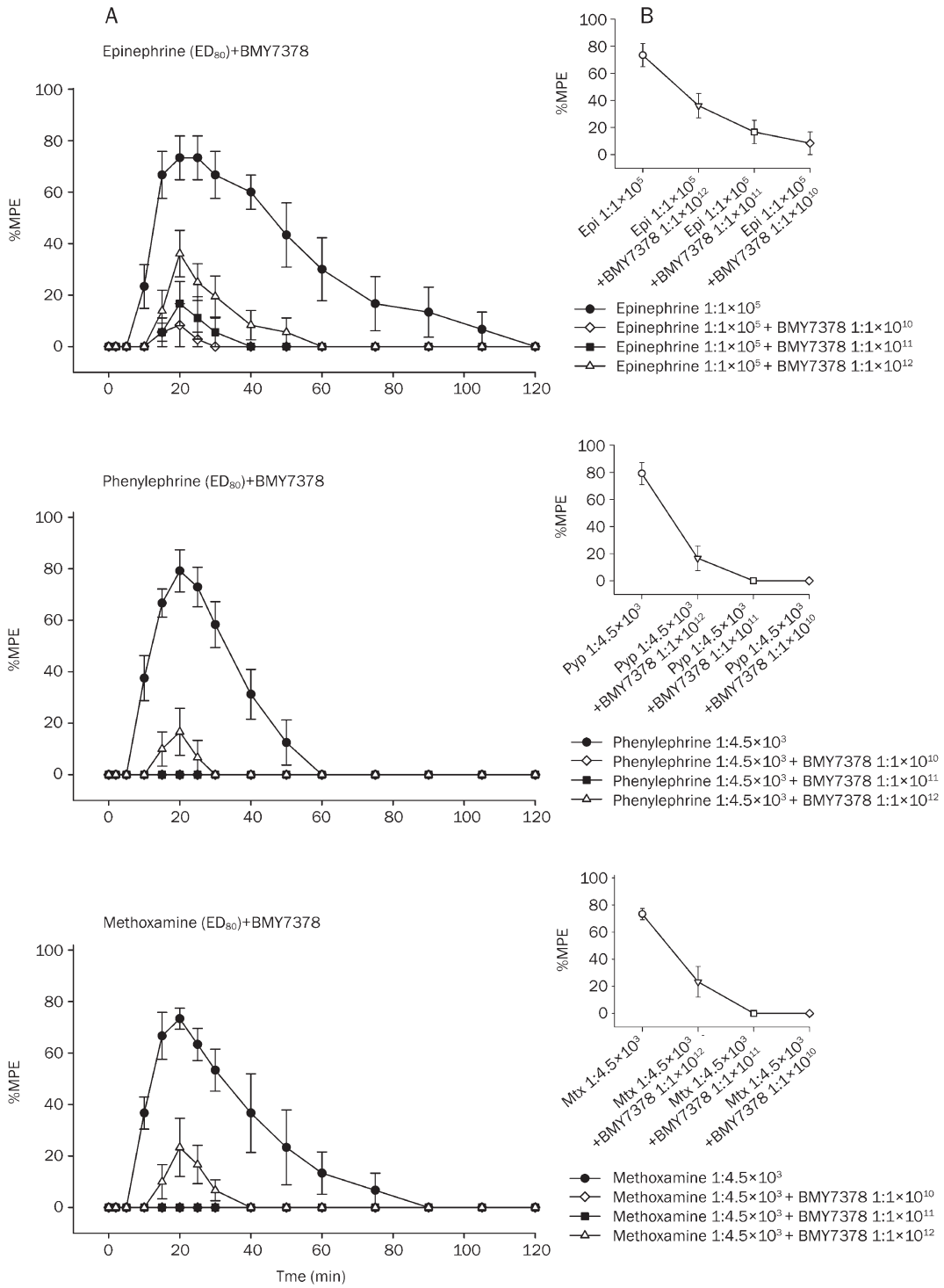

Figure 6. (A) Time courses of inhibition of cutaneous trunci muscle reflex by epinephrine, phenylephrine, and methoxamine in the presence of BMY7378 after subcutaneous injections of drugs in rats (n=6 rats for each drug). Values are mean±SEM. BMY7378 (0.3 mL) was injected 5 min before the injection of epinephrine (0.3 mL), phenylephrine (0.3 mL), and methoxamine (0.3 mL). Neurological evaluations were done before, and 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min after drug injection. (B) The dose-response curves of epinephrine, phenylephrine, and methoxamine in the presence of BMY7378 on cutaneous analgesia in rats (n=6 rats at each testing point). Values are mean±SEM and were fitted with the SASNLIN.

addition, the discrepancy between their and our results may be due to species difference.

Apparently, data from our results give cause to question the conventional wisdom described in the former section.

Table 2. Maximum possible effect of α-1 adrenoceptor agonist combined with NO donor on subcutaneous antinociception in rats.

		Vasodilator	%MPE±SEM
Epinephrine (ED_{80})	+	Nitroglycerin ($1:1\times10^{7}$)	0
		Nitroglycerin ($1:1\times10^{8}$)	0
		Nitroglycerin ($1:1\times10^{9}$)	0
Phenylephrine (ED_{80})	+	Nitroglycerin ($1:1\times10^{7}$)	0
		Nitroglycerin ($1:1\times10^{8}$)	0
		Nitroglycerin ($1:1\times10^{9}$)	0
Methoxamine (ED_{80})	+	Nitroglycerin ($1:1\times10^{7}$)	0
		Nitroglycerin ($1:1\times10^{8}$)	0
		Nitroglycerin ($1:1\times10^{9}$)	0
Epinephrine (ED_{80})	+	Nitroprusside ($1:1\times10^{7}$)	0
		Nitroprusside ($1:1\times10^{8}$)	0
		Nitroprusside ($1:1\times10^{9}$)	0
Phenylephrine (ED_{80})	+	Nitroprusside ($1:1\times10^{7}$)	0
		Nitroprusside ($1:1\times10^{8}$)	0
		Nitroprusside ($1:1\times10^{9}$)	0
Methoxamine (ED_{80})	+	Nitroprusside ($1:1\times10^{7}$)	0
		Nitroprusside ($1:1\times10^{8}$)	0
		Nitroprusside ($1:1\times10^{9}$)	0
Epinephrine (ED_{80})	+	Nifedipine ($1:1\times10^{7}$)	0
		Nifedipine ($1:1\times10^{8}$)	7±4
		Nifedipine ($1:1\times10^{9}$)	20±12
Phenylephrine (ED_{80})	+	Nifedipine ($1:1\times10^{7}$)	0
		Nifedipine ($1:1\times10^{8}$)	0
		Nifedipine ($1:1\times10^{9}$)	4±4
Methoxamine (ED_{80})	+	Nifedipine ($1:1\times10^{7}$)	0
		Nifedipine ($1:1\times10^{8}$)	0
		Nifedipine ($1:1\times10^{9}$)	8±5

Adrenoreceptor activation may affect various factors that regulate excitability, such as K^+ channel[30], Cl^- channel, or the Na^+-K^+ pump[31]. Epinephrine binding to α_1 and α_2 adrenoceptors of vascular smooth muscle causes vessel vasoconstriction, whereas epinephrine binding to β_2 receptors causes vasodilatation[8]. Percutaneously injected epinephrine will reach and vasoconstrict the vessels in the superficial epineural space first, and then penetrate into the nerve and the muscle. Vasoconstriction by epinephrine could result in a transient neural ischemia that directly induces nerve block[32]. Such an ischemia-induced nerve block may account for the local analgesia so apparent after injection of epinephrine, phenylephrine, or methoxamine[12, 13] (present results). The latency for this action, 15–20 min (Figure 1), was distinctly longer than that for almost immediate-acting, consistent with an accumulating reaction to ischemia or to a receptor-second messenger-mediated effect in neurons, rather than a direct local anesthetic action[33]. Our current data show that epinephrine and other α_1-adrenoceptor agonists cause local anesthesia, which can be blocked by treatment with nitric oxide donors (*eg*, nitroglycerin, nifedipine, and sodium nitroprusside) or Ca^{2+}-channel blocker (*eg*, nifedipine). This suggests that the local anesthetic activity of alpha-1 adrenoceptor agonists is due to nerve block resultant from neural ischemia mainly since it is expected that the effects of nitric oxide and nifedipine would oppose the effects of alpha-1 adrenoceptor agonists in both the nerve and vascular smooth. The contention is supported by many investigators. For example, epinephrine, clinically added to preparations of local anesthetics, prolonged the duration of action by reducing skin blood flow[34]. Adding epinephrine to lidocaine solutions increases the intensity and duration of sciatic nerve block in the rat[35]. By stimulating alpha-1 adrenoceptors on the neural vasculature[7], epinephrine mediates contraction of the vascular smooth muscle[8, 9], induces vasoconstriction, and thereby slows clearance of lidocaine from the nerve. Although systemic toxicity has not been reported to occur after subcutaneous infiltration of epinephrine, potential local toxicity such as delayed wound healing[36], increased wound infection rate[37], increased myocutaneous flap loss[38], and toxicity to skin[39] exists.

In summary, the current study provides the evidence to show that epinephrine and other α_1-adrenoceptor agonists can mainly act via mixed subtypes of α_1-adrenoceptor to induce local anesthetic activity.

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Author contribution

Ja-ping SHIEH and Mao-tsun LIN designed research; Ja-ping SHIEH and Chin-chen CHU performed research; Ja-ping SHIEH and Jhi-joung WANG contributed new analytical tools and reagents; Ja-ping SHIEH and Chin-chen CHU analyzed data; Mao-tsun LIN wrote the paper.

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