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Modification by Mepivacaine of the Contractile Responses to Transmural Electrical Stimulation and Exogenously Applied Norepinephrine in the Isolated Rabbit Aorta

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Abstract The effect of mepivacaine on the contractile responses to transmural electrical stimulation and exogenously applied norepinephrine (NE), potassium chloride (KCl) and histamine were studied in the isolated rabbit aorta. Mepivacaine, 5×10^{-5} to 5×10^{-4} M, attenuated the contractile response to transmural neural stimulation, the attenuation being greater in the response at high frequency stimulations. The attenuation of the responses by mepivacaine was not prevented by prior application of cocaine. The contractile response to norepinephrine (NE) was attenuated by mepivacaine, 5×10^{-5} to 2×10^{-3} M. The attenuation of the response to transmural stimulation was greater than that of the response to an equipotent concentration of exogenous NE. Pretreatment with mepivacaine, 5×10^{-5} to 2×10^{-3} M, protected alpha-adrenoceptors from persistent blockade by phenoxybenzamine in a dose-dependent manner. The contractile response to histamine was not significantly altered by mepivacaine in concentrations up to 5×10^{-4} M. Mepivacaine, 5×10^{-4} and 2×10^{-3} M, decreased the response to high concentrations of KCl. Ca^{2+}-induced contracion in aortic strips previously exposed to Ca^{2+} free media and depolarized by excess K^+ was significantly inhibited by mepivacaine, 5×10^{-4} and 2×10^{-3} M. It may be concluded that mepivacaine has alpha-adrenoceptor antagonistic action in addition to a sympathetic nerve conduction blocking action. Alpha-adrenoceptor antagonistic action is more evident in low concentration of mepivacaine than the Ca^{2+} antagonistic action.

Key Words: Local anesthetics; mepivacaine, sympathetic nervous system, Vascular smooth muscle; norepinephrine, transmural stimulation

Introduction

Mepivacaine is widely used for all types of infiltration and regional nerve block anesthesia. However, whether mepivacaine constricts[1,2] or dilates[3,5] the vascular smooth muscle is controversial. Åberg and Dhunér[6] reported that mepivacaine could produce vasodilation in the hind limb of dogs, when the vessels had previously been contracted by an infusion of norepinephrine (NE). In

contrast, if vascular tone was decreased by alphablockade, mepivacaine decreased the blood flow. According to Åberg and Wahlström[7], mepivacaine produced contractions of the isolated rat portal vein under relaxed conditions, while the compound relaxed the portal vein contracted with KCl and NE. It seems that the action of mepivacaine alters depending on the vasomotor tone of vascular smooth muscle. The major way in which neurogenic alterations of vasomotor tone are produced is undoubtedly through changes in the activity of sympathetic adrenergic nerves innervating the vascular wall[8]. Thus, the present study was undertaken to clarify the effect of mepivacaine on the response of isolated rabbit aortae to stimulation of sympathetic nerves and exogenously applied agonists, including NE, histamine and KCl, and to evaluate the actions of this compound on the adrenergic neuroeffector junction in the blood vessel wall.

Methods

Male albino rabbits weighing 1.8 to 2.5 Kg, anesthetized with ether, were killed by bleeding from the carotid arteries, and the thoracic aorta was isolated. The aorta was helically cut into strips approximately 25 mm long. The thoracic aorta was used for obtaining the contractile response to NE, KCl, and histamine. The specimen was fixed vertically between hooks, under a resting tention of 2 g, in a muscle bath (20 ml capacity) containing the nutrient solution. Hooks anchoring the upper end of the strips were connected to the lever of a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo, Japan). The solution was maintained at 37±0.5°C and aerated with a mixture of 95 per cent O_2 and 5 per cent CO_2. The composition of the nutrient solution was as follows (mM): Na^+ 143.0; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 153.9; HCO_3^- 25.0; SO_4^{2-} 1.2; $H_2PO_4^-$ 1.2; dextrose 10.0. The pH of the solution was 7.35 to 7.40. Before the start of experiments, the preparations were equilibrated for 60 to 90 minutes, during which time the bathing solution was replaced every 10 minutes.

The aortic strips were placed between stimulating electrodes of a platinum plate (5×10 mm)[9]. The gaps between the electrodes and strips were wide enough to allow for undisturbed contraction, and yet sufficiently narrow to permit effective stimulation of intramural nerve terminals. The preparations were transmurally stimulated by 0.3 msec square pulses with supramaximum intensity (20 V) at frequencies of 2, 5 and 20/sec. The number of electrical pulses was kept constant (200 pulses) by changing the period of stimulation (100, 40 and 10 seconds for frequencies of 2, 5 and 20/sec, respectively). Transmural stimulation was applied repeatedly untill steady responses were obtained. In five aortic strips, the effect of mepivacaine, 10^{-4} M, on the contractile responses to transmural stimulation in the presence of cocaine, 3×10^{-6} M, was tested. The effects of bretylium, 2×10^{-5} M or phentolamine, 10^{-6} M, on the contractile response to transmural stimulation were tested in four strips of each.

Norepinephrine, KCl and histamine were applied directly to the bathing medium in cumulative concentrations. After a 20 minutes exposure of preparations to test drugs, the dose-response relationships of NE, KCl and histamine and the contractile responses to transmural stimulation were obtained. The tention developed by NE, 5×10^{-5} M, KCl, 5×10^{-2} M, or histamine, 2×10^{-4} M, in control media was taken as 100 per cent.

To test the protection by mepivacaine from phenoxybenzamine-induced persistent blockade of alpha-adrenoceptors, the complete contractile response to NE was obtained first. In the non-treated series of experiments, preparations were left for 30 minutes in the medium without mepivacaine and then exposed for 30 minutes to phenoxybenzamine, 2×10^{-8} M. The preparations were washed with fresh nutrient solutions and equilibrated for 60 minutes. The contractile response to NE was then obtained. In the treated series of experiments, preparations were pretreated for 30 minutes with mepivacaine in various concentrations and for another 30 minutes with phenoxybenzamine, 2×10^{-8} M, in the presence of mepivacaine. After the treated drugs were discarded, the contractile response to NE was obtained as was in non-treated preparations.

Studies on the interaction between mepivacaine and Ca^{2+} were carried out as follows. The contractile response to KCl, 3×10^{-2} M, which was added to nutrient solutions, was obtained first, and the

strips were repeatedly washed and equilibrated for 60 minutes. Then, the preparations were exposed for 60 minutes to Ca^{2+} free media, during which time the medium was replaced twice every 20 minutes. Ten minutes after the addition of KCl (3×10^{-2} M) to the Ca^{2+} free media, Ca^{2+} in a concentration of 2.5 mM was added. When Ca^{2+} induced contractions stabilized, additional Ca^{2+} (2.5 and 5.0 mM) was applied. Preparations were treated for 20 minutes with mepivacaine before the addition of KCl.

Values presented in the text and figure are mean values ±SEM. The deta were analyzed statistically by the Student's paired or unpaired t test; $P<0.05$ was considered to be significant. Drugs used and their sources were: mepivacaine hydrochloride, Yoshitomi Pharmaceutical Co.; dl-norepinephrine hydrochloride, Sankyo Co.: histamine hydrochloride, Nakarai Chemical Ltd.; bretylium tosylate, Wellcome Pharmaceutical Co.; Phentolamine mesylate, Nippon Ciba-Geigy Ltd,; cocaine hydrochloride, Takeda Pharmaceutical Co; phenoxybenzamine hydrochloride, Nakarai Chemical Ltd.

Results

Treatment with mepivacaine, 2×10^{-5}M, did not alter the response to transmural stimulation. However, mepivacaine at 5×10^{-5}M significantly attenuated the response to the stimulation at 5 and 20/sec. Mepivacaine at 10^{-4}M significantly attenuated the response to the stimulation at all frequencies used, the attenuation being greater in the response at high frequencies. A further increase in the concentration to 5×10^{-4}M abolished the response to stimulation (Fig. 1).

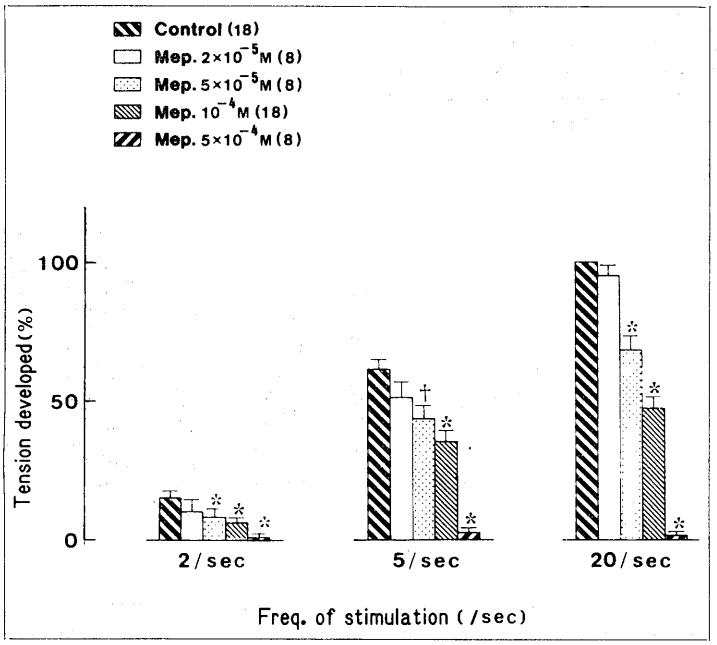

Fig. 1 Modification by mepivacaine of the contractile response to transmural stimulation. The response at a frequency of 20/sec in control media was taken as 100 per cent; the mean absolute value of the tension was 0.60±0.05 g (n=18). Figures in parenthesis indicate the number of preparations used. Mep.=mepivacaine. *=$p<0.001$, †=$p<0.05$ for differences from control. Mepivacaine attenuated the tension developed at higher frequencies to a greater extent.

Table 1 Modification of Mepivacaine-induced Attenuation of the Contractile Response to Transmural Stimulation by Cocaine.

Frequencies of stimulation	Solution		Solution		
	Control	Mepivacaine 10^{-4}M	Control	Cocaine 3×10^{-6}M	Mepivacaine 10^{-4}M
5/sec	0.36±0.06g (100) (n=5)	0.23±0.08g (57)* (n=5)	0.33±0.04g (n=5)	0.73±0.13g (100)* (n=5)	0.39±0.14g (49)† (n=5)
20/sec	0.59±0.09g (100) (n=5)	0.21±0.07g (33)* (n=5)	0.52±0.08g (n=5)	1.09±0.12g (100)* (n=5)	0.26±0.09g (22)†* (n=5)

Figures in parenthesis represent percentage of the tension (values obtained prior to mepivacaine were taken as 100 per cent).
*; $P<0.05$, compared to data for control : paired t test
†; $P<0.05$, compared to data for cocaine group : paired t test.
n; number of preparations.

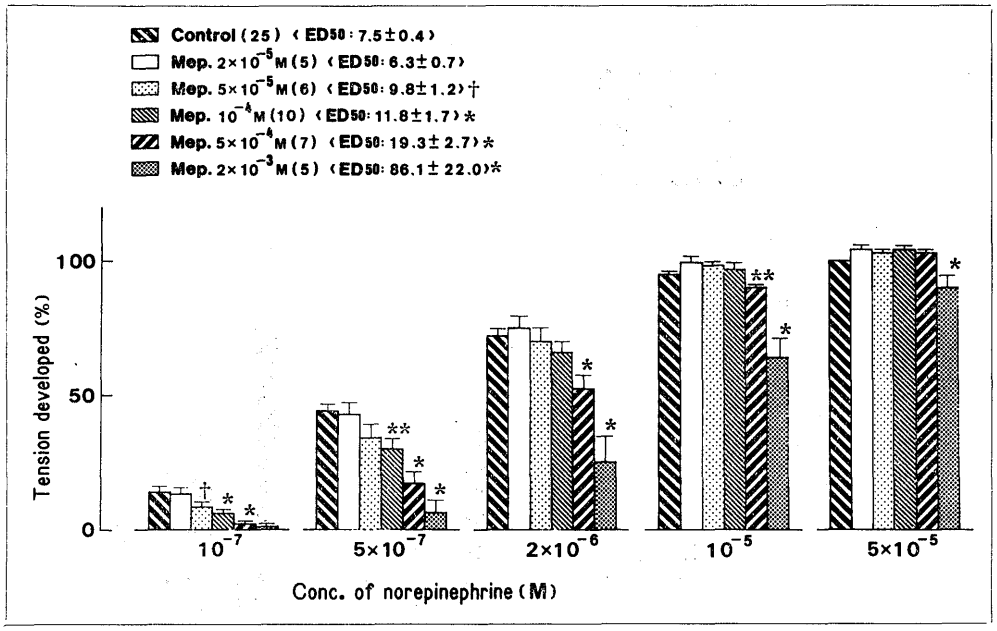

Fig. 2 Effects of mepivacaine on the contractile responses to norepinephrine (NE) and their ED50 ($\times10^{-7}$M) values. The contractile response to NE, 5×10^{-5}M, in control media was taken as 100 per cent; the mean absolute value of the tension was 2.48±0.12g (N=25). *=$p<0.001$, **=$p<0.01$, †=$p<0.05$ for differences from control. Mepivacaine attenuated the contractile responses to NE in a dose-dependent manner and their respective ED50 values were increased.

The inhibition was reversed by repeated washing of preparations. The inhibitory effect of mepivacaine, 10^{-4}M, was not prevented by treatment with cocaine, 3×10^{-6}M, which inhibited NE uptake in sympathetic nerve terminals[10] (Table 1). The tension of aortic strips was not altered by mepivacaine in concentrations up to 2×10^{-3}M. Contractile responses to transmural stimulation were abolished by treatment for 20 minutes with bretylium, 2×10^{-5}M, phentolamine, 10^{-6}M, in all of four aortic strips of each.

The contractile responses to NE were attenuated by mepivacaine (5×10^{-5} to 2×10^{-3}M) in a dose-dependent manner and their respective median effective concentration (ED 50) values were increased (Fig. 2).

The inhibitory effect was reversed by repeated washing of the preparations. Concentrations of NE sufficient to produce the same magnitude of contractions as that with transmural stimulation at a frequency of 20/sec was 3.4×10^{-7}M. Mepivacaine, 5×10^{-5}, 10^{-4}M and 5×10^{-4}M, reduced the response to this concentration of NE by $5.1\pm3.3\%$ (N=6), $11.0\pm2.9\%$ (N=10), and $24.8\pm4.5\%$ (N=7), respectively, whereas inhibitions of the responses to transmural stimulation at 20/sec by these concentrations of mepivacaine averaged $32.0\pm4.2\%$ (N=5), $57.3\pm3.3\%$ (N=18), and $99.8\pm0.2\%$ (N=8), respectively.

Treatment with phenoxybenzamine, 2×10^{-8}M, markedly attenuated the contractile response to NE (Fig. 3, non-treated). Prior treatment with mepivacaine prevented the inhibitory effect of phenoxybenzamine (Fig. 3, compare non-treated and mepivacaine-treated preparations). The higher was the concentration of mepivacaine, the greater

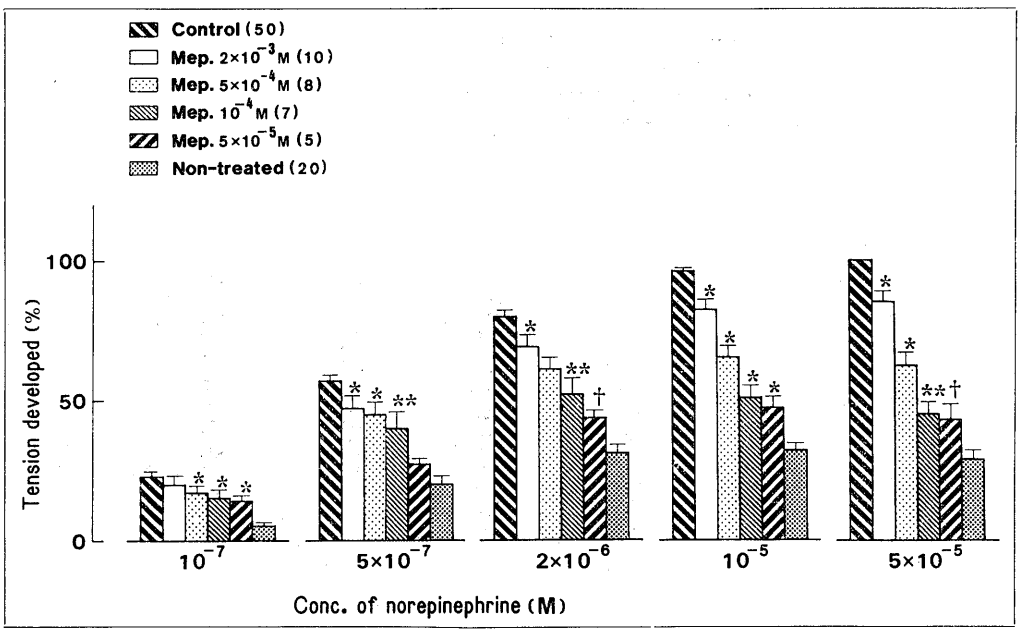

Fig. 3 Modification of phenoxybenzamine-induced alpha blockade by prior treatment with mepivacaine. The contractile response to NE, 5×10^{-5}M, in control media before treatment with mepivacaine and phenoxybenzamine was taken as 100 per cent; the mean absolute value of the contraction was 2.74 ± 0.09 g (N=50). Non-treated; Contractile response to NE in preparations which were not pretreated with mepivacaine but treated with phenoxybenzamine, 2×10^{-8}M.

the prevention was (Fig. 3).

Mepivacaine in concentrations lower than 10^{-4} M did not alter the dose-response to KCl, but at 5×10^{-4}M reduced the contraction induced by high concentrations of KCl (30 to 50 mM). The greater attenuation was attained after treatment with 2×10^{-3}M mepivacaine (Fig. 4).

ED50 values were not altered by mepivacaine up to 5×10^{-4}M. Treatment with mepivacaine at 2×10^{-3}M attenuated the contractions with histamine and significantly increased the ED50 values (Fig. 5).

Contractions induced by Ca^{2+} (2.5 mM) in aortic strips previously exposed for 60 minutes to Ca^{2+} free media and depolarized by excess K^+ were inhibited by mepivacaine at 5×10^{-4} and 2×10^{-3}M in a dose-dependent manner. The inhibitory effect of mepivacaine was overcome by raising external Ca^{2+} to 5.0 and 7.5 mM (Fig. 6).

Discussion

The contractile response of isolated rabbit aortae to transmural electrical stimulation under experimental condition used in the present study is considered to result from NE released by excitation of adrenergic nerves, since the responses were abolished by an alpha-adrenoceptor blocking agent (phentolamine), an adrenergic neuron blocking agent (bretylium) or tetrodotoxin in the present and previous studies[9,10,11]. Mepivacaine attenuated the responses to transmural neural stimulation and to exogenously applied NE in a dose-dependent manner. Bevan and Su[12] have postulated that a uniform distribution of exogenous and non-uniform (high concentration close to nerve terminals, the

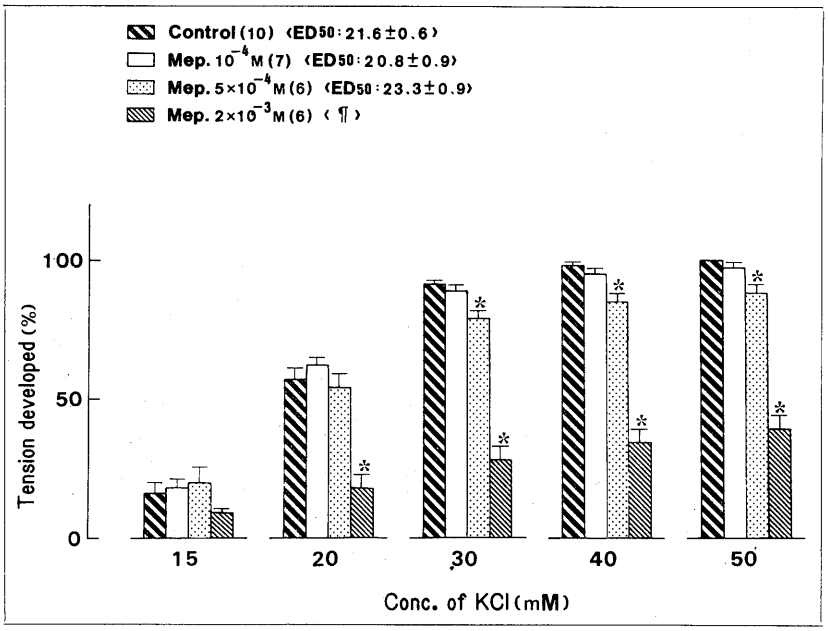

Fig. 4 The effects of mepivacaine on the contractile responses to potassium chloride (KCl) and their ED50 ($\times10^{-5}$M) values. The contractile response to KCl, 5×10^{-2}M, in control media was taken as 100 per cent; the mean absolute value of the tension was 2.39±0.08 g (n=10). *=P<0.001 for differences from control Mepivacaine in concentrations lower than 10^{-4}M did not alter the response to KCl.

further the distance from the nerves, the less is the concentration of NE) distribution of neurogenic NE exist throughout the medium. When equal responses to neurogenic and exogenous NE are induced, the concentration at the nerve terminals of neurogenic NE must be higher than that of the exogenous NE. Thus, the well known alpha-adrenoceptor blocking agents like phentolamine or phenoxybenzamine reduce the response to exogenous NE more effectively than the response to adrenergic nerve stimulation[12]. In the present study, however, the attenuation of the response to adrenergic nerve stimulation by mepivacaine, 5×10^{-5} to 5×10^{-4} M, was greater than that of the response to an equipotent concentration of exogenous NE. Therefore, it may be concluded that mepivacaine interferes with the release of NE from adrenergic nerves in addition to the action on alpha-adrenoceptors.

The interference with the release of NE may be caused either by a bretylium like action or by a blockade of nerve conduction. In the present study, prior application of cocaine in a concentration sufficient to prevent the inhibitory effect of bretylium on the release of NE[10] did not prevent mepivacaine-induced attenuation. In addition, the response to high frequencies of transmural stimulation was attenuted by mepivacaine to a greater extent. Similar results were obtained with lidocaine[9]. Such an uneven effectiveness may be related to reduced excitability and impaired conduction of nerves by local anesthetics. These findings suggest that mepivacaine attenuates the contractile response to transmural stimulation

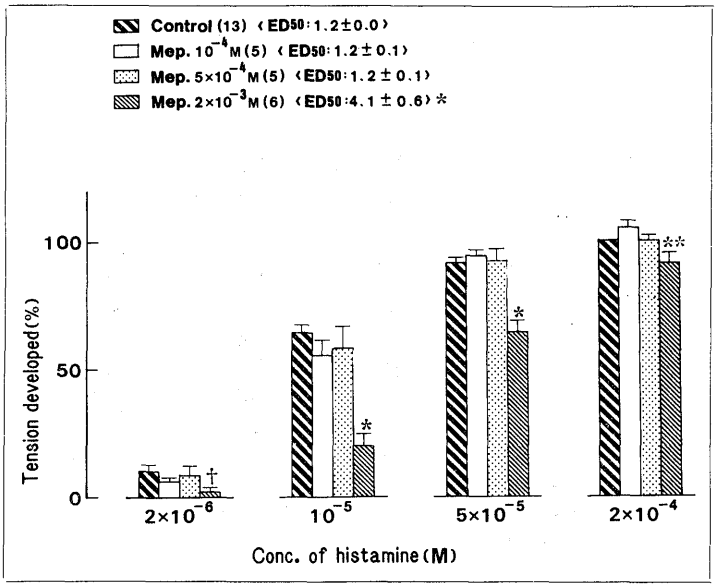

Fig. 5 The effects of mepivacaine on the contratile responses to histamine and their ED50 ($\times10^{-3}$M) values. The contractile response to histamine, 2×10^{-4}M, in control media was taken as 100 per cent; the mean absolute value of the tension was 2.54±0.13 g (n=13). *=P< 0.001, **=P<0.01, †=P<0.05 for differences from control. Mepivacaine in concentrations from 2×10^{-5}M did not alter the response to histamine.

due to the nerve conduction blockade rather than to an action like bretylium.

Mepivacaine, in concentrations insufficient to reduce the contractile response to KCl and histamine, attenuated the contractile responses to NE and increased their respective ED 50 values in rabbit aortic strips. Furthermore, treatment of aortic strips with mepivacaine effectively protected alpha-adrenoceptors from persistent blockade of phenoxybenzamine. Only blocking agents of a competitive type are effective in such a receptor protection[13]. These findings indicate that mepivacaine reversibly and competitively antagonizes alpha-adrenoceptors. It has been well known that local anesthetics have Ca^{2+} antagonistic action[14]. In the present study, high concentrations of mepivacaine attenuated the response to KCl or to Ca^{2+} in aortic strips previously exposed to Ca^{2+} free media and the attenuation of Ca^{2+} -induced contractions by mepivacaine was reversed by excess Ca^{2+}. Thus, it seems likely that the influx of Ca^{2+} across aortic cell membrane is inhibited by high concentration of mepivacaine. It must be emphasized that the alpha-adrenoceptor antagonistic action is more evident in low concentrations of mepivacaine than the Ca^{2+} antagonistic action.

Mepivacaine did not alter the base-line tension of the aortic strips. Findings similar to the present study were presented by Al-

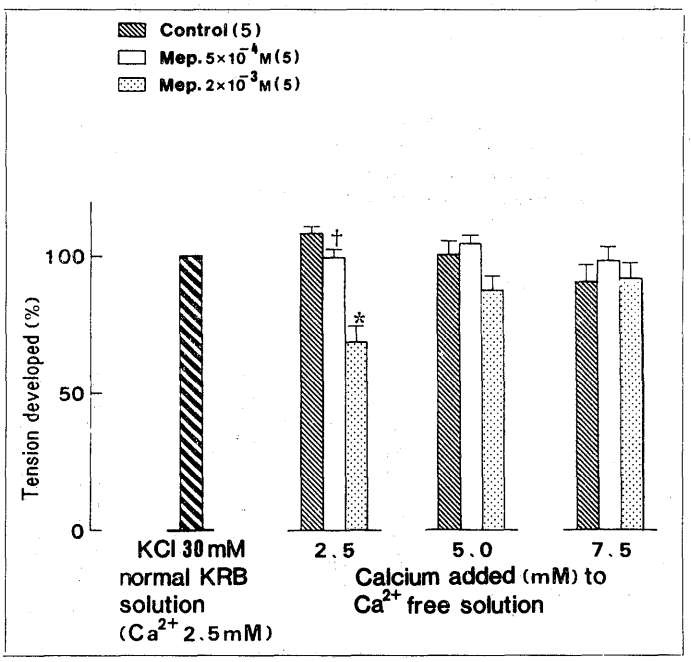

Fig. 6 The effects of mepivacaine on calcium induced contractions in preparations exposed to Ca^{2+} free media containing excess KCl (3×10^{-2}M). The contractile response to KCl, 3×10^{-2}M, in normal solutions (Ca^{2+} 2.5mM) was taken as 100 per cent; the mean absolute value of the tension was 2.22 ± 0.07 g (n=15). *=$P<0.001$, †=$P<0.05$, for differences from control. The calcium (2.5mM)-induced contraction was attenuated by high concentrations of mepivacaine. Excess calcium (5.0 and 7.5 mM) restored the contraction.

tura and Altura[15]. They found that mepivacaine in concentrations to 10^{-3}M did not contract isolated arteries from dogs, rats, rabbits and cats. However, Åberg and Wahlström[7] reported that mepivacaine produced contractions of the isolated rat portal vein under relaxed condition. The difference of responsiveness to mepivacaine between arteries and veins under relaxed condition was not readily explained by the present study.

The plasma concentration of mepivacaine reaches to 10 μg/ml (4×10^{-5}M) at the maximum in intercostal nerve block without signs of systemic toxicity[16]. In this study, the contractile responses of isolated aorta to transmural stimulation and low concentrations of NE were significantly attenuated by mepivacaine, 5×10^{-5} M. Furthermore, it has been stated that in vitro aortic strips may be less sensitive to the blocking agent than in vivo microvascular smooth muscle[17]. Thus, mepivacaine in clinical doses is expected to cause arterial vasodilation through the alpha-adrenoceptor antagonism in addition to the sympathetic nerve conduction blockade.

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References

1) Jorfeldt, L., Lofström, B., Pernow, B. and Wahren, J.: The effect of mepivacaine and lignocaine on forearm resistance and capacitance vessels in man. *Acta Anaesthesiol. Scand.*, 14 : 183–201, 1970.

2) Adler, R., Adler, G. and Åberg, G.: Studies on dental blood flow in dogs. *Svensk. Tandlak. Tidskr.*, 62 : 699–703, 1969.

3) Dhunér, K.G. and Lewis, D.H.: Effect of local anaesthetics and vasoconstrictors upon regional blood flow. *Acta Anaesthesiol. Scand.*, 10 : 347–352, 1966.

4) Nishimura, N., Morioka, T., Sato, S. and Kuba, T.: Effect of local anesthetic agents on the peripheral vascular system. *Anesth. Analg.*, 44 : 135–139, 1965.

5) Blair, M.R.: Cardiovascular pharmacology of local anesthetics. *Br. J. Anaesth.*, 47 : 247–252, 1975.

6) Åberg, G. and Dhunér, K.G.: Effects of mepivacaine (Carbocaine ®) on femoral blood flow in the dog. *Acta Pharmacol. Toxicol.*, 31 : 267–272, 1972.

7) Åberg, G. and Wahlström, B.: Mechanical and electrophysiological effects of some local anaesthetic agents and their isomers on the rat portal vein. *Acta Pharmacol. Toxicol.*, 31 : 255–266, 1972.

8) Vanhoutte, P.M.: Microcirculation: Heterogeneity in vascular smooth muscle. Baltimore, University Park Press, 1978, p. 189.

9) Fukuda, S, Takeshita, H. and Toda, N.: Modification by lidocaine and its n-dealkylated metabolites of the isolated rabbit aorta to transmural stimulation. *Anesthesiology*, 53 : 106–112 1980.

10) Toda, N.: Interaction of bretylium and drugs that inhibit the neuronal membrane transport of norepinephrine in isolated rabbit atria and aortae. *J. Pharmacol. Exp. Ther.*, 181 : 318–327 1972.

11) Paterson, G.: The response to transmural stimulation of isolated arterial strips and its modification by drugs. *J. Pharm. Pharmacol.*, 17: 341–349, 1965.

12) Bevan, J.A. and Su, C.: Distribution theory of resistance of neurogenic vasoconstriction to alphareceptor blockade in the rabbit. *Cir. Res.*, 28 : 179–187, 1971.

13) Ariëns, E.H.: Molecular pharmacology: The mode of action of biologically active compound. New York and London, Academic Press, 1964, p. 412.

14) de Jong, R.H.: Local anesthetics: Direct effects of vascular wall. Springfield, Illinois, Charles C Thomas. 1977, p. 131.

15) Altura, B.M., Altura, B.T.: Effects of local anesthetics, antihistamines, and glucocorticoids on peripheral blood flow and vascular smooth muscle. *Anesthesiology*, 41 : 197–214, 1974.

16) Tucker, B.T., Moore, D.C., Bridenbaugh, P.O., Bridenbaugh, L.D., Thompson, G.E.: Systemic absorption of mepivacaine in commonly used regional block procedures. *Anesthesiology*, 31 : 277–287, 1972.

17) Toda, N., Hatano, Y.,: Alpha-adrenergic blocking action of fentanyl on the isolated aorta of the rabbit. *Anesthesiology*, 46 : 411–416, 1977.