Bull Yamaguchi Med Sch 28 : 89-97, 1981

Effects of Lidocaine and Its N-Dealkylated Metabolite on the Response of Isolated Rabbit Aorta to Transmural Stimulation

Satoru Fukuda

Department of Anesthesiology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

(Received May 2, 1981)

Abstract Contractile responses of rabbit aortic strips to transmural stimulation and to exogenously applied norepinephrine (NE), potassium chloride (KCl), and histamine were studied in the presence of lidocaine or its metabolite, glycinexylidide (GX). Lidocaine, 10^{-4} and 5×10^{-4}M, attenuated the contractile response to transmural stimulation. GX, 2×10^{-5} to 5×10^{-4}M, potentiated the response to transmural stimulation. The suppression induced by lidocaine was not reversed by excess calcium, 2.2 and 4.4 mM, but was partially reversed by cocaine, 3×10^{-6}M. Lidocaine, 5×10^{-4}M shifted the dose-response curve of NE to the right, whereas GX, 5×10^{-4}M, shifted the curve to the left. The maximum tension developed by K^+ was attenuated by lidocaine, 5×10^{-4}M, and GX, 2×10^{-3}M. It may be condcluded that lidocaine attenuates the response to stimulation of sympathetic nerves innervating the arterial wall by interfering with the release of NE. In contrast, GX potentiates the response, possibly by increasing the release of NE.

Key Words: Anesthetics; lidocaine. Lidocaine; sympathetic nerve, metabolites. Smooth muscle; vessels, lidocaine, norepinephrine, sympathetic nerve

Introduction

Lidocaine is degraded into several metabolites, of which glycinexylidide (GX) have been stated to have the same antiarrhythmic [1,2], local anesthetic[3,4], and central nervous system actions[2,4,5] as lidocaine. Modification by lidocaine of vascular reactivity to vasoconstricting agents in vitro has been investigated by Fleisch and Titus[6] and by Altura and Altura[7]. Further studies of the effects of the pharmacologically active metabolite, GX, may provide data for better understanding of circulatory changes induced by injections of lidocaine in vivo. The lack of knowledge about the effects of these metabolite on vascular smooth muscle, and the wide use of lidocaine in clinical practice as antiarrhythmic and a local anesthetic, prompted us to examine the effects of GX on the responses of the isolated rabbit aorta to transmural stimulation and to exogenous application of norepinephrine (NE), potassium chloride (KCl), and histamine. The effects of GX were compared with those of lidocaine.

Methods and Materials

Male albino rabbits, 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 ascending aorta was used for studies of transmural stimulation and the descending aorta was used for obtaining the dose-response curves of NE, KCl, and histamine. The specimen was fixed vertically between hooks, under a resting tension of 2 g, in a muscle bath (20 ml capacity) containing the nutrient solution. Hooks anchoring the upper end of the strip were connected to the lever of a force-displacement transducer (Nihonkoden 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^+ 162.1; K^+ 5.4; Ca^{++} 2.2; Cl^- 157.0; HCO_3 14.9; dextrose 5.6. The pH of the solution was 7.2 to 7.3. Before the start of experiments, the preparations were equilibrated for 60 to 90 min, during which time the bathing solution was replaced every 10 min.

Stimulating electrodes of platinum plate (5×10 mm) were placed parallel to each other on both sides of the aortic strip. The gaps between the electrode and the strip were wide enough to allow for undisturbed contraction, and yet sufficiently narrow to permit effective stimulation of intramural nerve terminals[8]. The intrinsic sympathetic nerve terminals that remained in the aortic wall were stimulated by 0.3-msec square pulses with supramaximum intensity (20 V) at frequencies of 2, 5, and 20/s. The number of electrical pulses was kept constant (200 pulses) by changing the period of stimulation (100, 40, and 10 s for frequencies of 2, 5, and 20/s, respectively). Transmural stimulation was applied repeatedly until steady responses were obtained. In four preparations the response to transmural stimulation was tested in the presence of phentolamine, 10^{-6}M or bretylium, 2×10^{-5}M. The effects of Ca^{++}, 2.2 and 4.4mM, or cocaine, 3×10^{-6}M, on the response to transmural stimulation in the preparation treated

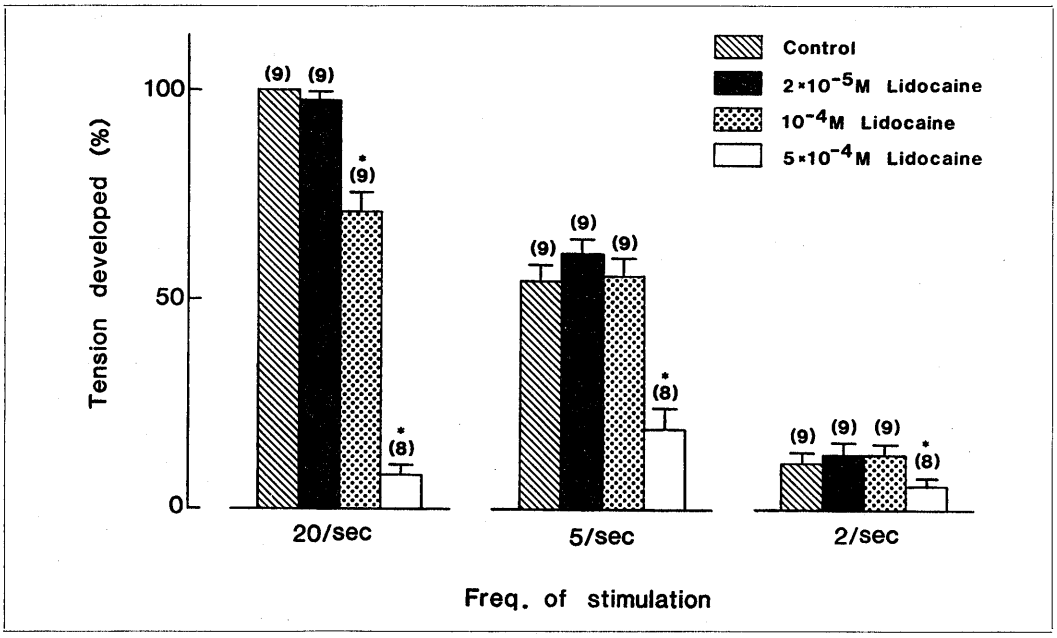

Fig. 1 Modification by lidocaine of the response to transmural stimulation. The response at a frequency of 20/s in control medium was taken as 100 per cent. Mean value of the tension developed at 20/s in control medium was 0.46 ± 0.06g (n = 9). *indicates significant difference from control. Parenthesis shows number of experiment. Subsequent figures have similar characteristics.

with lidocaine, or GX, 5×10^{-4}M, were studied. In seven preparations the response to transmural stimulation by cocaine, 3×10^{-6}M, alone, was tested It was ascertained that the altered responses of the preparation treated with lidocaine or its metabolite were returned to the control state by repeated washing of preparations.

Cumulative dose-response curves for NE, KCl, and histamine were obtained in both the absence (control) and the presence of 2×10^{-5} to 2×10^{-3}M lidocaine, or GX. After 20 min exposure of preparations to test drugs, the responses to transmural stimulation or the dose-response relationship for NE, KCl, or histamine were obtained. The tension developed by NE, 5×10^{-5}M, KCl, 5×10^{-2}M, or histamine, 2×10^{-4}M, in control medium was taken as 100 per cent. Values presented in the text and figures are mean values ± SE. The data were analyzed statistically by the Student t test unpaired for data; $P<0.05$ was considered significant.

Results

Contractile responses of aortic strips to transmural stimulation were abolished by pretreatment with bretylium, 2×10^{-5}M, and phentolamine, 10^{-6}M, in four of four preparations. The resting tension of aortic strips was not altered by lidocaine, or GX in concenteations $\leq 2\times10^{-3}$M.

Treatment with lidocaine, 2×10^{-5}M, did not alter the response to transmural stimulation at frequencies of 2, 5, and 20/s, whereas lidocaine, 10^{-4}M, significantly attenuated the response to transmural stimulation at 20/s. Further increase in the concentration to 5×10^{-4}M suppressed the response to stimulation at all frequencies used (Fig. 1). The inhibitory effect of lidocaine was reversed by repeated washing of preparations. Treatment with GX in concentrations ranging

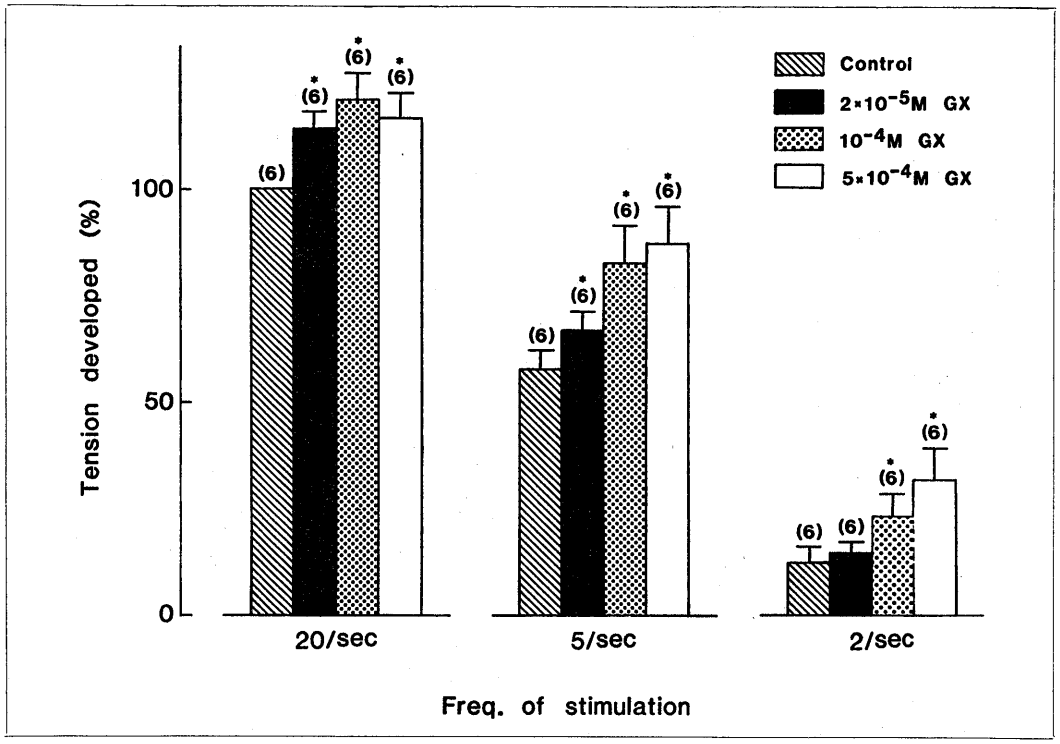

Fig. 2 Modification by GX of the response to transmural stimulation. Mean value of the tension of contraction at 20/s in control medium was 0.47 ± 0.07g (n = 6).

from 2×10^{-5} to 5×10^{-4}M significantly potentiated the response to transmural stimulation (Fig. 2). The potentiating effect was reversed by repeated washing of preparations.

The inhibitory effect of lidociane on the response to transmural stimulation was not affected by the addition of Ca^{++}, 2.2 and 4.4 mM. Cocaine, 3×10^{-6}M, incompletely reversed the inhibitory effects of lidociane, 5×10^{-4}M, at 20/s, but significantly stimulated the response to transmural stimulation over the control at 2/s (Fig. 3).

Treatment with cocaine alone significantly potentiated the response to transmural stimulation, and combined treatment with cocaine, 3×10^{-6}M, and GX, 5×10^{-4}M, caused an additional increase in the response (Fig. 4).

The dose-response curve of NE was shifted to the right by lidocaine, 5×10^{-4}M and, in contrast, was shifted to the left by GX, 5×10^{-4}M (Table 1). Concentrations of NE sufficient to produce the same magnitude of contractions as that with transmural stimulation at frequencies of 5 and 20/s were 5.5×10^{-8} and 9.5×10^{-8}M, respectively Lidocaine, 5×10^{-4}M, reduced the responses to these concentrations of NE by 11.6 ± 9.6 and 9.6 ± 7.0 per cent (n=5), respectively; this represented markedly less than average inhibitions of the responses to transmural stimulation at 5 and 20/s (65.9 ± 9.6 and 92.9 ± 2.2 per cent, n=8, respectively).

Effects of lidocaine and GX in concentrations from 2×10^{-5} to 2×10^{-3}M on the maximum contractions induced by NE, histamine

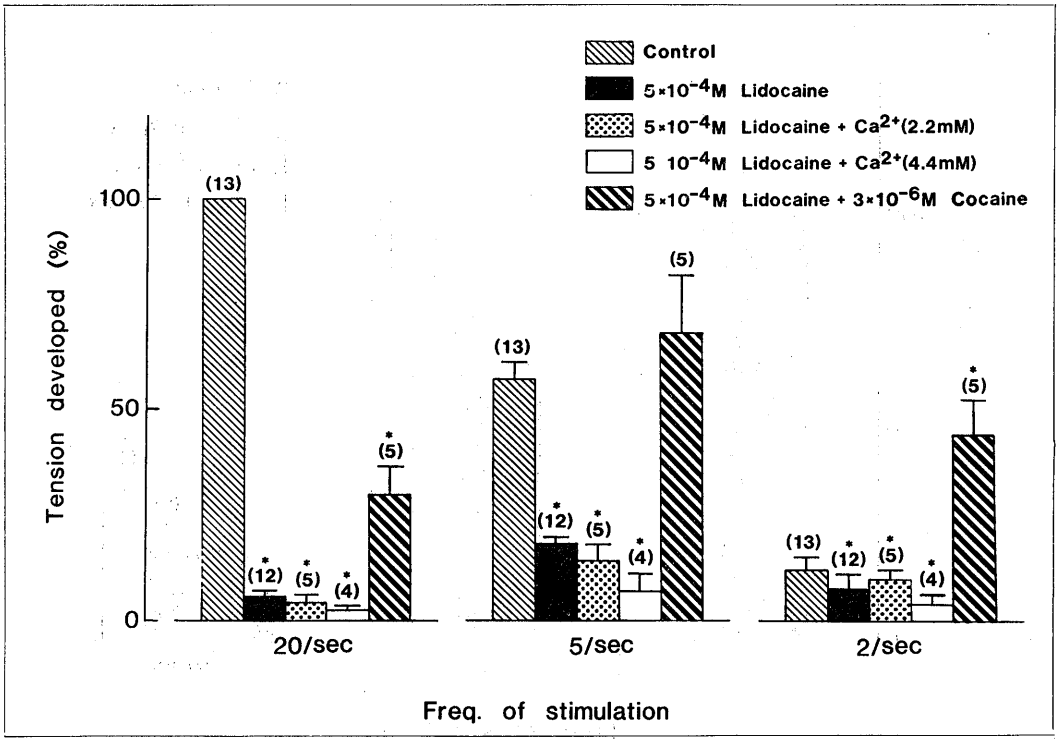

Fig. 3 Modification by Ca^{++}, 2.2 and 4.4mM, and cocaine, 3×10^{-6}M, of the inhibitory effects of lidocaine at 5×10^{-4}M on the tension developed. Mean value of the contraction in control medium was 0.44 ± 0.03g (n=13).

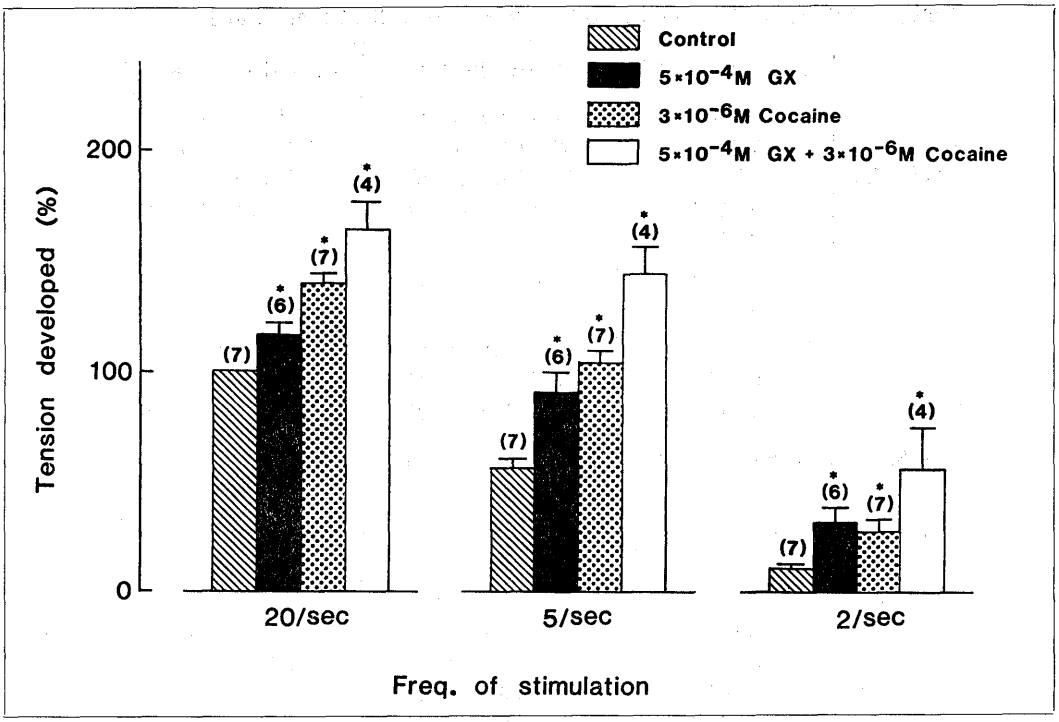

Fig. 4 Modification by cocaine, 3×10^{-6}M, of the potentiating effects of GX at 5×10^{-4}M on the tension developed. Mean value of the contraction at 20/s in control medium was 0.28±0.04g (n = 7).

Table 1 Effects of Lidocaine and GX on Responses of Rabbit Aorta to Norepinephrine

	Concentration (M)	ED_{50} ($\times10^{-7}$M)	Maximal response (%)
Lidocaine	control	3.6±0.5 (n=11)	100(n=11)
	2×10^{-5}	3.8±0.5 (n=7)	100.7±1.3 (n=7)
	10^{-4}	3.6±0.5 (n=6)	99.8±2.1 (n=6)
	5×10^{-4}	5.8±0.8*(n=5)	102.2±0.9 (n=5)
	2×10^{-3}	—	—
GX	control	3.9±0.3 (n=13)	100(n=13)
	2×10^{-5}	4.1±0.3 (n=6)	100.4±0.6 (n=6)
	10^{-4}	3.4±0.7 (n=4)	101.8±0.3 (n=4)
	5×10^{-4}	2.4±0.2†(n=5)	102.5±1.7*(n=5)
	2×10^{-3}	4.2±0.8 (n=5)	97.6±2.7 (n=5)

*Different from control, $P<0.05$, †Different from control, $P<0.01$.

Table 2 Effects of Lidocaine and GX on Responses of Rabbit Aorta to KCl

	Concentration (M)	ED_{50} ($\times10^{-3}$M)	Maximal response (%)
Lidocaine	control	21.5±0.9 (n=8)	100(n=8)
	2×10^{-5}	20.5±1.0 (n=5)	101.6±1.3 (n=5)
	10^{-4}	22.3±1.3 (n=5)	101.5±3.3 (n=5)
	5×10^{-4}	23.7±0.6 (n=4)	85.1±1.2† (n=4)
	2×10^{-3}	—	—
GX	control	20.6±1.0 (n=7)	100(n=7)
	2×10^{-5}	—	—
	10^{-4}	19.1±0.9 (n=6)	100.8±2.0 (n=6)
	5×10^{-4}	20.6±2.3 (n=4)	96.9±1.8 (n=4)
	2×10^{-3}	23.0±1.8 (n=4)	82.6±1.3† (n=4)

*Different from control, $P<0.05$, †Different from control, $P<0.01$.

Table 3 Effects of Lidocaine and GX on Responses of Rabbit Aorta to Histamine

	Concentration (M)	ED_{50} ($\times10^{-5}$M)	Maximal response (%)
Lidocaine	control	5.2±0.6 (n=10)	100(n=10)
	2×10^{-5}	5.5±0.9 (n=5)	102.6±2.1 (n=5)
	10^{-4}	5.2±0.8 (n=5)	104.8±4.8 (n=5)
	5×10^{-4}	5.4±0.9 (n=4)	102.8±4.6 (n=4)
	2×10^{-3}	21.6±2.3† (n=5)	94.3±2.9† (n=5)
GX	control	5.6±0.6 (n=10)	100(n=10)
	2×10^{-5}	—	—
	10^{-4}	5.0±0.8 (n=4)	99.7±2.3 (n=4)
	5×10^{-4}	4.3±0.5 (n=5)	101.7±0.6 (n=5)
	2×10^{-3}	4.9±0.8 (n=5)	102.1±2.2 (n=5)

*Different from control, $P<0.05$, †Different from control, $P<0.01$.

and K^+, and the ED50s of these agents, are listed in Tables 1-3. The maximum response to K^+ was attenuated by lidocaine, 5×10^{-4}M, and GX, 2×10^{-3}M. The inhibitory effect was greater with lidocaine.

Discussion

The contractile response to transmural stimulation applied under experimental conditions used in the present study is considered to result from NE released by excitation of adrenergic nerves, since the response is abolished by alpha-adrenoceptor blocking agents, adrenergic neuron blocking agents, or tetrodotoxin[8-10]. Lidocaine, 10^{-4}M, in concentrations insufficient to attenuate the contractile response to exogenous NE, reduced the response to transmural stimulation, and the attenuation of the response to transmural stimulation by lidocaine, 5×10^{-4}M, was appreciably greater than that of the response to exogenous NE. In contrast, it is known that alpha-adrenoceptor blocking agents reduce the response to exogenous NE more effectively than the response to adrenergic nerve stimulation[11]. It may therefore

be concluded that the attenuation by lidocaine appears to be due mainly to an interference with the release of NE from adrenergic nerve terminals.

That the inhibitory effect of lidocaine was related directly to frequencies of transmural neural stimulation, as seen with frog sciatic nerve[13], may indicate the involvement of de-pression of nerve action potentials in the interference with the release of NE. Extracellular Ca^{++} affects the membrane-stabilizing effect of local anesthetics on isolated lobster axons[14], but does not influence the effect on myelinated nerves or squid axons[15,16]. Thus, the finding that the addition of Ca^{++} (2 and 3 times normal) to isolated aortic strips did not reverse the inhibitory effect of lidocaine does not necessarily exclude the possibilijy that the local anesthetic stabilizes the autonomic nerve membrane. Further, inhibition of the transmembrane influx of Ca^{++} may not be involved in the depressant effect of lidocaine.

As shown in Figure 1, the tension developed at higher frequencies was more susceptible to lidocaine. This is specific to local anesthetics, as reported by Covino and Vassallo[13]. Toda[9] reported that the tension developed at 5 or 20/s was attenuated by bretylium, and that this inhibitory effect was reversed with resulting potentiation by cocaine. Such potentiation can be explained either by active extrusion of bretylium accumulated within nerve terminals or by some other mode of inactivation of the bretylium by cocaine. Failure to demonstrate potentiation or reversal at 5 and 20/s by cocaine in the presence of lidocaine may be due to more susceptibility of the tension developed to lidocaine at higher frequencies. These considerations suggest that lidocaine possesses a bretylium-like inhibitory action in addition to action specific to local anesthetics at the nerve treminals.

Treatment with GX, 2×10^{-5}, 10^{-4}, and 5×10^{-4}M, potentiated the response to transmural stimulation. Similar potentiation of the responses to transmural stimulation and to exogenous NE has been shown with cocaine desipramine, and pyrogallol, but not with monoamine oxidase inhibitiors[17]. In the present study, dose-response curves of NE were not significantly influenced by GX, 2×10^{-5} and 10^{-4}M, which were sufficient to potentiate the response to transmural stimulation. Furthermore, the potentiating effects of GX and cocaine were additive. Cocaine in the concentration used here (3×10^{-6}M) is sufficient to produce the maximum potentiation in association with an inhibition of neuronal uptake of NE[17]. These findings suggest that the potentiation induced by GX is related to an increase in the release of NE from nerve terminals, rather than an amine-uptake inhibition. In the highest concentration used (5×10^{-4}M), GX potentiated the response to exogenous NE, suggesting that this concentration interferes with inac tivation processes of NE.

According to Hudgins et al[18]., vasoconstricting agents, including NE, histamine, and KCl, interact with Ca^{++} via different mechanisms. Fleisch and Titus[6] have postulated that lidocaine blocks α-adrenoceptors in rat aortic strips. However, in the present study, we could not demonstrate an α-blocking action of lidocaine. The response is not necessarily attributable to α-adrenergic blockade, because lidocaine, 5×10^{-4}M, also attenuated the response to K^{+}.

Increases of systemic vascular resistance during intravenous infusion of lidocaine have been reported to occur in man[19-22] and in the dog[20,23]. In this study, lidocaine, 2×10^{-5} M (4.68 μg/ml), which is close to plasma levels attained for therapy of arrhythmias in clinical practice[24], did not affect the tension developed. Plasma concentrations of GX during lidocaine infusion in man reach as high as 4.5 μg/ml (2.6×10^{-5}M)[25]. These concentrations were proved to be sufficient to significantly potentiate the contractile response of arterial smooth muscle to adrenergic nerve stimulation in this study.

Therefore, an increased vascular resistance could be explained, at least in part, by an increased release of NE at the nerve terminals by GX. The present study suggests that the metabolite play a significant role in changing cardiovascular function during lidocaine infusion in vivo.

This work is a thesis for the Graduate School of Medicine, Yamaguchi University.

References

1) Burney, R.G., Difazio, C.A., Peach, M.J., et al.: Anti-arrhythmic effects of lidocaine metabolites *Am. Heart J.*, 88 : 765-769, 1974.
2) Smith, E.R., Duce, B.R.: The acute antiarrhythmic and toxic effects in mice and dogs of 2-ethylamino-2′, 6′-acetoxylidine (L-86), A metabolite of lidocaine. *J. Pharmacol. Exp. Th -er.*, 179 : 580-585, 1971.
3) Ehrenberg, L.: The time-concentration curve of local anesthetics. *Acta Chem. Scand.*, 2 : 63-81, 1948.
4) Strong, J.M., Parker, M., Atkinson, A.J. Jr.: Identification of glycinexylidide in patients treated with intravenous lidocaine. *Clin. Pharmacol. Ther.*, 14 : 67-72, 1973.
5) Blumer, J., Strong, J.M., Atkinson, A.J. Jr.: The convulsant potency of lidocaine and its N-dealkylated metabolites. *J. Pharmacol. Exp. Ther.*, 186 : 31-36, 1973.
6) Fleisch, J.H., Titus, E.: Effect of local anesthetics on pharmacologic receptor systems of smooth muscle. *J. Pharmacol. Exp. Ther.*, 186 : 44-51, 1973.
7) 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.
8) Toda, N., Usui, H., Mori, J.: Contractile responses of spiral strips of large blood vessels from rabbits to trransmural stimulation and tyramine. *Jpn. J. Pharmacol.*, 22 : 59-69, 1972.
9) Toda, N.: Interactions 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.
10) Paterson, G. : The response to transmural stimulation of isolated arterial strips and its modification by drugs. *J. Pharm. Pharmacol.*, 17 : 341-349, 1965.
11) Bevan, J.A., Su, C.: Distribution theory of resistance of neurogenic vasoconstriction to alpha-receptor blockade in the rabbit. *Circ. Res.*, 28 : 179-187, 1971.
12) Heavner, J.E., de Jong, R.H.: Lidocaine blocking concentrations for B-and C-nerve fibers. *Anesthesiology*, 40 : 228-233, 1974.
13) Covino, B.G., Vassallo, H.G.: Local anesthetics, mechanism of action and clinical use. New York, Grune and Stratton, 1976, p. 45.
14) Blaustein, M.P., Goldman, D.E.: Competitive action of calcium and procaine on lobster axon *J. Gen. Physiol.*, 49 : 1043-1063, 1966.
15) Narahashi, T., Frazier, D.R., Takeno, K.: Effects of calcium on the local anesthetic suppression of ionic conductances in squid axon membranes. *J. Pharmacol. Exp. Ther.*, 197 : 426-438, 1976.
16) Arhem, P., Frankenhaeuser, B.: Local anesthetics: Effects on permeability properties of nodal membrane in myelinated nerve fibers from Xenopus. Potential clamp experiments. *Acta Physiol. Scand.*, 91 : 11-21, 1974.
17) Toda, N.: Influence of cocaine and desipramine on the contractile response of isolated rabbit pulmonary arteries and aortae to transmural stimulation. *J. Pharmacol. Exp. Ther.*, 179 : 198-206, 1971.
18) Hudgins, P.M., Weiss, G.B.: Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmacol. Exp. Ther.*, 159 : 91-97, 1968.
19) Klein, S., Sutherand, R.I.L., Morch, J.: Haemodynamic effects of intravenous lignocaine in man. *Can. Med. Assoc. J.*, 99 : 472-475, 1968.
20) Jorfeldt, L., Lofström, B., Pernow, B., et al.: The effect of local anaesthetics on the central circulation and respiration in man and dog. *Acta Anaesthesiol. Scand.*, 12 : 153-169, 1968.
21) Boudoulas, H., Schaal, S.F., Lewis, R.P., et al.: Negative inotropic effect of lidocaine in patients with coronary arterial disease and normal subjects. *Chest*, 71 : 170-175, 1977.
22) McWhirter, W.R., Frederickson, E.L., Steinhaus, J.E.: Interactions of lidocaine with general anesthetics. *South Med. J.*, 65 : 796-800, 1972.
23) McWhirter, W.R., Schmidt, F.H., Frederickson

E.L., et al.: Cardiovascular effects of controlled lidocaine overdosage in dogs anesthetized with nitrous oxide. *Anesthesiology*, 39 : 398–404, 1973.
24) Blair, M.R.: Cardiovascular pharmacology of local anaesthetics. *Br. J. Anaesth.*, 47 : 247–252, 1975.
25) Strong, J.M., Mayfield, D.E., Atkinson, A.J. Jr. et al.: Pharmacological activity, metabolism and pharmacokinetics of glycinexylidide. *Clin. Pharmacol. Ther.*, 17 : 184–194, 1974.