Mechanisms of Action of Eperisone on Isolated Dog Saphenous Arteries and Veins

Seiji INOUE, Ka BIAN, Tomio OKAMURA, Hideki OKUNISHI and Noboru TODA
Department of Pharmacology, Shiga University of Medical Sciences, Seta, Ohtsu 520-21, Japan
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Abstract—Effects of eperisone, an antispasmodic in skeletal muscle, were investigated in helical strips of dog saphenous artery and vein. Eperisone relaxed saphenous arteries and veins previously contracted with norepinephrine, serotonin, acetylcholine, K+ or Ba2+: but in contrast, it produced contractions in the blood vessels contracted with prostaglandin (PG) F_2α. Treatment with eperisone attenuated the contractions induced by norepinephrine and serotonin in the arteries and those by clonidine and phenylephrine in the veins. Eperisone inhibited angiotensin II-induced relaxations, mediated possibly by endogenous PGI_2, but did not alter relaxations caused by exogenous PGI_2. Treatment with eperisone (10^{-5} M) potentiated the contractile response to electrical stimulation of adrenergic nerves; the potentiating effect was suppressed by yohimbine. The eperisone-induced contraction in PGF_2α-contracted arteries was inhibited by treatment with indomethacin or aspirin, although cyclooxygenase activity was not inhibited by eperisone. These results may indicate that eperisone blocks postjunctional α₁- and α₂-adrenergic, muscarinic, serotoninergic receptors and prejunctional α₂-adrenoceptors and reduces PGI_2 synthesis via a mechanism other than cyclooxygenase inhibition.

Eperisone hydrochloride (4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride) possesses antitremorine and antinicotinic actions (1) and inhibits mono- and multisynaptic reflexes by suppressing α- and γ-efferent neuron activities in the spinal cord and supraspinal structures (2). The drug is clinically used to relieve skeletal muscle stiffness. Recent studies indicate an eperisone-induced increase in blood flow in the skeletal muscle of anesthetized rabbits (personal communication from Dr. M. Arai) and dogs (3).

The agents possessing the piperidinopropiophenone structure relax visceral and vascular smooth muscles (4-7), possibly due to a direct action on smooth muscle, like that of papaverine (6, 7). Fujioka and Kuriyama (8) have reported that eperisone has Ca²⁺ antagonistic actions in isolated guinea pig basilar artery. Our preliminary study showed that eperisone did not relax but contracted the isolated dog saphenous artery and vein previously contracted with PGF_2α indicating that its action is different from those of papaverine and Ca²⁺ antagonists.

Therefore, the present study was undertaken to determine the action and the mechanisms of action of eperisone on receptor-mediated responses of various agents in isolated saphenous arteries and veins, skeletal muscle vasculatures. Abilities of this drug to inhibit contractions mediated by α-adrenergic, serotoninergic and muscarinic receptors and to potentiate contractions due to adrenergic nerve stimulation were demonstrated.

Materials and Methods

Male and female mongrel dogs, weighing 7-13 kg, were anesthetized with intravenous injections of sodium pentobarbital (30 mg/kg) and killed by bleeding from the carotid arteries. Saphenous arteries and veins and interlobar branches of the renal artery were quickly removed and cut helically into approximately
20-mm long strips. The strips were fixed vertically between hooks in a muscle bath of 20-ml capacity containing the modified Ringer-Locke solution, which was maintained at 37±0.3°C and aerated with a mixture of 95% O₂ and 5% CO₂. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo, Japan). Resting tensions for the artery and vein strips were adjusted to 1.5 g and 0.7 g, respectively, which are optimal for inducing maximum contractions. The solution had the following composition: 120 mM NaCl, 5.4 mM KCl, 25.0 mM NaHCO₃, 2.2 mM CaCl₂, 1.0 mM MgCl₂ and 5.6 mM dextrose. The pH of the solution was 7.3-7.4. Before the start of experiments, the strips were allowed to equilibrate for 60 to 90 min in control media, during which time the solutions were replaced every 10-15 min.

Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Nihon Kohden Kogyo Co.). Contractile responses to 30 mM K⁺ were first obtained; and in vein strips, contractions induced by 5 mM Ba²⁺ were also obtained. Responses to agonists were obtained under resting conditions or in strips partially contracted with various agents, the contraction being in a range between 20-45% of the contraction induced by 30 mM K⁺ in the arteries and by 5 mM Ba²⁺ in the veins. Contractions induced by agonists were presented as values relative to those induced by 30 mM K⁺ in the arteries and by 5 mM Ba²⁺ in the veins. Relaxations were presented as values relative to those induced by 10⁻⁴ M papaverine. Cumulative concentration-response curves for agonists were obtained by adding the drugs directly to the bathing media. Preparations had been treated for 20-30 min with blocking agents before the concentration-response curve for agonists was obtained. Endothelium was removed by gently rubbing the intimal surface with cotton pellets. The endothelial function was determined by testing the relaxant response to acetylcholine. Renal artery strips were used only for experiments with angiotensin II, which produced relaxations, possibly by the release of PG₁₂ (9).

To test the protection by eperisone from phenoxybenzamine-induced persistent blockade of α adrenoceptors (10) in saphenous artery strips, the norepinephrine concentration-response curve was first obtained in normal media. In the non-treated series of experiments, the artery strips were left for 30 min in the medium without eperisone and then exposed for 30 min to 2x10⁻⁸ M phenoxybenzamine. The strips were repeatedly washed with fresh medium and equilibrated for 60 min. The concentration-response curve for norepinephrine was then obtained. In the treated series of experiments, the strips were pretreated for 30 min with 10⁻⁴ M eperisone and then treated for an additional 30 min with eperisone plus phenoxybenzamine. Paired comparisons were made in strips obtained from the same dogs.

Saphenous artery strips were placed between a pair of stimulating electrodes made of platinum plates, 5x15 mm in size and approximately 2 mm apart from each other. The gap between the strip and electrodes was wide enough to allow undisturbed contractions, and yet sufficiently narrow to permit stimulation of intramural nerve terminals effectively. The strips were transmurally stimulated by a train of 0.3 msec square pulses of supramaximum intensity (approximately 10 V), at frequencies of 2, 5 and 20 Hz for periods of 100, 40 and 10 sec respectively. Stimulations at 5 Hz were applied repeatedly with an interval of 10 min until steady state responses were obtained.

Isotope experiments were carried out on helical strips of dog saphenous arteries, as previously described (11). Briefly, the tissue was preincubated for 60 min at 37°C with 0.5 M [³H]-norepinephrine (specific activity, 43.9 Ci/mmol). It was then superfused with the modified Ringer-Locke solution containing cocaine (3x10⁻⁶ M) and corticosterone (4x10⁻⁶ M) at a rate of 1 ml/min. The preincubated strips were stimulated electrically four times for 3 min at a frequency of 5 Hz. Stimulation periods started after 126 (S₁), 144 (S₂), 162 (S₃) and 180 min (S₄) of superfusion. The stimulation-evoked overflow of total tritium was calculated by subtraction of basal overflow. Eperisone was added 12 min before S₄. The effect of eperisone on the stimulation-evoked ³H-overflow was express-
ed as the ratio between the overflow evoked by $S_4$ and that evoked by $S_3$. The ratio was compared with that obtained in the absence of treatment with eperisone.

Two helical strips of the saphenous artery obtained from the same dogs were used for the paired analysis of PGF$_{2\alpha}$ and 6-keto PGF$_{1\alpha}$ contents in the bathing media. After a 10-min preincubation at 37°C, the paired strips (one of which was treated with $10^{-5}$ M eperisone and the other left untreated) were exposed for 15 min to arachidonic acid ($10^{-7}$ M); then the medium was acidified with 0.5 ml of 1 N HCl to pH 3.0, and indomethacin ($10^{-6}$ M) was added in order to stop the further production and degradation of PG products. The bathing medium was quickly collected by aspiration and stored frozen at below -60°C until the assay. Samples were concentrated ten times by a SEP-PAK C18 column (Waters Associates, Milford, MA, U.S.A.) prior to the assay. PGF$_{2\alpha}$ and 6-keto PGF$_{1\alpha}$ were measured by radioimmunoassay using commercial kits (PGF$_{2\alpha}$ and 6-keto PGF$_{1\alpha}$ [3H] assay systems, Amersham, England).

The effect of eperisone on cyclooxygenase activity was examined in comparison with that of indomethacin, according to the procedure reported by Miyamoto et al. (12). Briefly, the reaction mixture (100 µl) consisted of 0.1 M Tris-HCl buffer at pH 8.0, 2 nM hematin, 5 mM tryptophane, 40 nM [1-14C]arachidonic acid, cyclooxygenase from sheep vesicular glands (5.7 µg of protein) and eperisone (10^{-6} to 10^{-3} M) or indomethacin (10^{-10} to 10^{-5} M). Following a 5 min-preincubation at 24°C, the reaction was started by the addition of [1-14C]arachidonic acid and carried out at 24°C for another 5 min. The medium was extracted with 300 µl of citrated ether, and a 100-µl aliquot of the organic phase was placed on a silica gel plate (E. Merck, Darmstadt, F.R.G.), which was then developed for 50 min at -20°C. The radioactivity on the plates was measured by a chromatoscanner.

The results shown in the text, table, and figures are expressed as mean values±S.E.M. Statistical analyses were made using Student's paired and unpaired t-test and Tykey's method after one-way analysis of variance. Drugs used were eperisone (4'-ethyl-2'-methyl-3'-piperidinopropiophenone hydrochloride; E-0646. Eisai Co., Tokyo, Japan); prostaglandin F$_{2\alpha}$ and PG$_{1\alpha}$ sodium salt (Ono Pharmaceutical Co., Osaka, Japan); serotonin creatinine sulfate (E. Merck AG, Darmstadt, F.R.G.); acetylcholine chloride (Daichichi Pharmaceutical Co., Tokyo); clonidine hydrochloride, (±)-phenylephrine hydrochloride, arachidonic acid (AA) sodium salt, corticosterone and indomethacin (Sigma Chemical Co., St. Louis, MO, U.S.A.); aspirin and yohimbine hydrochloride (Nakarai Chemicals, Ltd., Kyoto, Japan); phenoxylbenzamine hydrochloride (Smith Kline & French Labs., Philadelphia, PA, U.S.A.); substance P and angiotensin II (Peptide institute, Inc., Minoh, Japan); papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka); atropine sulfate (Tanabe Seiyaku Co., Ltd., Osaka); ketanserin tartrate (Kyowa Hakko Co., Tokyo); tetrodotoxin, cocaine hydrochloride and (±)-norepinephrine (Sankyo Co., Ltd., Tokyo); (−)-ring-2,5,6-[3H]norepinephrine (NEN, Boston, MA, U.S.A.); and sodium pentobarbital (Abbott Lab., North Chicago, IL, U.S.A.).

Results

Effect of eperisone on saphenous artery and vein strips contracted with various agents: In helical strips of the dog saphenous artery and vein partially precontracted with PGF$_{2\alpha}$, the addition of eperisone in concentrations ranging from $10^{-7}$ to $10^{-3}$ M produced concentration-related contractions (Figs. 1 and 2). In the artery strips contracted with K+, eperisone in concentrations of $10^{-5}$ and $10^{-4}$ M produced slight contractions, but the vein strips contracted with K+ or Ba$^{2+}$ responded to $10^{-4}$ M eperisone with a relaxation. On the other hand, eperisone (10^{-6} to $10^{-4}$ M) produced slight contractions, but the vein strips contracted with K+ or Ba$^{2+}$ responded to $10^{-4}$ M eperisone with a relaxation. The other hand, eperisone (10^{-6} to $10^{-4}$ M) relaxed dose-dependently both saphenous artery and strips partially contracted with norepinephrine or serotonin (Figs. 1 and 2). Eperisone relaxed saphenous vein strips contracted with acetylcholine (Fig. 2) to a greater extent than those contracted with norepinephrine or serotonin. Serotonin-induced contractions were suppressed by $10^{-6}$ M ketanserin, and acetylcholine-induced contractions in the veins
Fig. 1. Typical recordings of the response to eperisone of a saphenous artery strip contracted with PGF$_{2a}$, norepinephrine (NE), or serotonin (5-HT). Concentrations of eperisone (Ep.) from 7 to 4=$10^{-7}$ to $10^{-4}$ M, respectively; PA=$10^{-4}$ M papaverine. Horizontal lines just left of each tracing represent the level prior to the addition of drug.

Fig. 2. Typical recordings of the response to eperisone of a saphenous vein strip contracted with PGF$_{2a}$, acetylcholine (ACh), Ba$^{++}$, or norepinephrine (NE). Concentrations of eperisone (Ep.) from 7 to 4=$10^{-7}$ to $10^{-4}$ M, respectively; PA=$10^{-4}$ M papaverine.
Modification by eperisone of the response to vasoactive agents: In dog saphenous arteries, contractions induced by norepinephrine were attenuated by treatment with eperisone (10^{-5} M and 10^{-4} M) dose-dependently (Fig. 4, left). In the artery strips treated for 30 min with 2 \times 10^{-8} M phenoxybenzamine and repeatedly washed, the contractile response to norepinephrine was markedly attenuated (Fig. 5, left). However, when the arteries were treated with 10^{-4} M eperisone plus phenoxybenzamine, the attenuation of the response to norepinephrine was appreciably less (Fig. 5, right) than that of the response of arteries treated with phenoxybenzamine alone (Fig. 5, left). Eperisone (10^{-5} and 10^{-4} M) also inhibited the contraction caused by serotonin and the relaxation due to acetylcholine in a dose-dependent manner (Fig. 4, middle and right). Acetylcholine-induced relaxations were markedly suppressed by removal of endothelium. Inhibitions by eperisone of the response to acetylcholine were appreciably greater than those of the response to norepinephrine and serotonin. Relaxations by substance P (10^{-7} M) were dependent on the endothelium in the presence of 10^{-5} M indomethacin, and they were markedly inhibited by 10^{-7} M atropine.

Quantitative data on saphenous artery and vein strips contracted with a variety of vasoconstrictors are summarized in Fig. 3.

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Quantitative data on saphenous artery and vein strips contracted with a variety of vasoconstrictors are summarized in Fig. 3.
Fig. 4. Modification by eperisone (10^{-5} and 10^{-4} M) of the response of saphenous artery strips to noradrenaline (left panel), serotonin (middle), and acetylcholine (right). Contractions induced by 10^{-5} M noradrenaline or 10^{-5} M serotonin in control media were taken as 100% (for left and middle panels); mean absolute values were 3256±251 mg (n=6) and 3180±556 mg (n=7), respectively. Relaxations induced by 10^{-4} M papaverine were taken as 100% (for right panel); mean absolute values in control and eperisone (10^{-5} and 10^{-4} M)-containing media were 642±38 mg (n=6), 690±99 mg (n=6) and 713±121 mg (n=6), respectively. Significantly different from the control: *P<0.01. Significantly different from values obtained with 10^{-5} M eperisone: †P<0.01. Numbers in parentheses indicate the number of preparations used. Vertical bars represent S.E.M.

were not influenced by treatment with eperisone (10^{-5} and 10^{-4} M); mean values of the relaxations in control and eperisone (10^{-5} and 10^{-4} M)-treated arteries were 73.4±5.1% (n=5), 76.2±5.5% (n=5) and 70.3±6.4% (n=4), respectively. The relaxations induced by PGI2 in saphenous arteries were not influenced by treatment with eperisone; mean values of the relaxation due to 10^{-8} M PGI2 in control and eperisone (10^{-5} and 10^{-4} M)-treated strips were 33.0±3.8% (n=6), 31.8±4.7% (n=6) and 33.3±2.7% (n=4), respectively.

In order to determine possible actions of eperisone on arachidonic acid metabolism, experiments were carried out with isolated dog renal arteries, which respond to angiotensin II with a relaxation, possibly via the release of PGI2 from the arterial wall (9, 13). The addition of angiotensin II (10^{-7} M) to renal artery strips contracted with PGF2α produced a slight contraction followed by a moderate relaxation. The relaxant response (58.7±6.1%, n=9) was not altered by treatment with 10^{-5} M eperisone (57.0±8.0%, n=9), but was significantly attenuated at 10^{-4} M (35.0±7.5%, n=9, P<0.05). Eperisone (10^{-4} M) did not inhibit the relaxation by PGI2 (n=9). The peptide-induced relaxation was reversed to a contraction by treatment with 10^{-6} M indomethacin (n=4).

Saphenous veins, but not saphenous arteries, responded to clonidine with contractions, which were attenuated by treatment with 10^{-8} M yohimbine. Contractile responses of saphenous vein strips to clonidine and phenylephrine were attenuated by eperisone (10^{-6} to 10^{-4} M) (Fig. 6). The inhibition was reversed by repeated washing of the preparations.

Modification by eperisone of the contractile response and the 3H-overflow caused by transmural stimulation: Transmural electrical stimulation applied at frequencies of 2, 5 and 20 Hz for periods of 100, 40 and 10 sec, respectively, produced a frequency-dependent
Fig. 5. Modification by phenoxybenzamine (POB, 2×10^{-8} M, left) and eperisone (Ep., 10^{-4} M) plus phenoxybenzamine (right) of the concentration-response curve for norepinephrine in saphenous arteries. Solid circles=concentration-response curves for norepinephrine obtained in normal media; open circles=the curves obtained after repeated washing of preparations treated with phenoxybenzamine alone or with eperisone plus phenoxybenzamine. See the Methods for details. Contractions induced by 10^{-5} M norepinephrine in normal media were taken as 100%; mean absolute values in experiments with phenoxybenzamine alone (left) and with eperisone plus phenoxybenzamine (right) were 3069±611 mg (n=6) and 3598±755 mg (n=6), respectively. Significantly different from the control: aP<0.001, bP<0.005, cP<0.02, dP<0.05. Numbers in parentheses indicate the number of the preparations used. Vertical bars represent S.E.M.

 contraction in saphenous artery strips, which was abolished by 3×10^{-7} M tetrodotoxin. The addition of eperisone (10^{-7} to 10^{-4} M) did not change the arterial tone. Treatment with eperisone in concentrations of 10^{-6} and 10^{-5} M potentiated the contractile response to transmural stimulation (Fig. 7), whereas 10^{-4} M eperisone almost completely abolished the contraction elicited at 5 Hz stimulation. The results are summarized in Table 1. Treatment with yohimbine in a concentration of 3×10^{-9} M also potentiated the contractile response to 5 Hz stimulation (26.6±7.9% increase, n=5). In yohimbine-treated strips, eperisone (3×10^{-6} M) did not significantly potentiate the contraction elicited at 5 Hz stimulation (2.4±1.9% increase, n=5), whereas in control strips from the same dogs, the stimulation-induced contraction was increased by 19.2±1.4% (n=5).

Transmural electrical stimulation was also applied at a frequency of 5 Hz for 3 min to saphenous artery strips previously soaked for 60 min in a bathing medium containing 5×10^{-7} M 3H-norepinephrine and superfused for 2 hr in the control medium. The ratio of 3H-overflow (S4/S3) was significantly increased by treatment with eperisone in a concentration of 10^{-5} M; average S4/S3 values in control and eperisone-treated strips were 0.94±0.051 (n=4) and 1.22±0.061 (n=6, P<0.02), respectively.

Effects of eperisone, indomethacin and aspirin on saphenous artery strips: In saphenous artery strips partially contracted with PGF2α, the addition of indomethacin (10^{-8} to 10^{-6} M) or aspirin (2×10^{-6} to 5×10^{-5} M) produced concentration-dependent contractions, as did eperisone (10^{-6} to 10^{-4} M) (Fig. 8). In the strips pretreated with indomethacin (10^{-6} M) or aspirin (5×10^{-5} M), eperisone (10^{-6} and 10^{-5} M)-induced contractions
Fig. 6. Modification by eperisone (Ep., $10^{-6}$, $10^{-5}$ and $10^{-4}$ M) of the contractile response of saphenous vein strips to clonidine (left panel) and phenylephrine (right). Contractions induced by $10^{-5}$ M clonidine or $10^{-4}$ M phenylephrine in control media were taken as 100%; mean absolute values were 1005±173 mg (n=7) and 960±122 mg (n=8), respectively. Significantly different from the control: aP<0.01, bP<0.05. Significantly different from the values at $10^{-6}$ M eperisone: cP<0.01. Significantly different from the values at $10^{-5}$ M eperisone: dP<0.01. Numbers in parentheses indicate the number of the preparations used. Vertical bars represent SEM.

Table 1. Modification by eperisone of contractile responses to transmural electrical stimulation in saphenous artery strips

| Treatment       | n  | 2 Hz | 5 Hz | 20 Hz |
|-----------------|----|------|------|-------|
| None            | 9  | 110±37 mg (100%) | 345±82 mg (100%) | 616±101 mg (100%) |
| Eperisone $10^{-7}$ M | 5  | 111±7.0% | 106±4.6% | 104±2.8% |
| Eperisone $10^{-6}$ M | 9  | 151±16.8% | 124±6.2% | 114±4.2% |
| Eperisone $10^{-5}$ M | 8  | 204±24.3% | 151±15.9% | 116±4.1% |
| Eperisone $10^{-4}$ M | 6  | 4.7±2.1% | |

Significantly different from the controls (no treatment): aP<0.01, bP<0.05. Significantly different from the values obtained with $10^{-7}$ M eperisone: cP<0.01, dP<0.05. Significantly different from the values obtained with $10^{-6}$ M and $10^{-5}$ M eperisone: eP<0.01 and fP<0.01, respectively.

were abolished (Fig. 8).

In order to determine whether eperisone inhibits the activities of cyclooxygenase and/or PG12 synthase, PGF2α and 6-keto PGF1α produced from arachidonic acid in saphenous arteries were measured. Mean values of PGF2α and 6-keto PGF1α were 17.3±4.0 and 54.4±20.1 (pg/mg wt.) (n=4), respectively, in control strips and 17.1±4.5 and 57.2±19.1 (pg/mg wt.) (n=4), respectively, in the strips treated with $10^{-5}$ M eperisone; the difference in the values in the absence and presence of
Fig. 7. Potentiation by eperisone (Ep.) of the response of a saphenous artery strip to transmural electrical stimulation at frequencies of 2, 5 and 20 Hz. TTX=tetrodotoxin.

Fig. 8. Concentration-response curves for indomethacin (left panel), aspirin (right) and eperisone in saphenous artery strips contracted with PGF$_2\alpha$. X=data with eperisone in the arteries pretreated with $10^{-6}$ M indomethacin (Indo., left) or $5 \times 10^{-5}$ M aspirin (Asp., right). Contractions induced by 30 mM K$^+$ were taken as 100% contraction; mean absolute values were 2750±610 mg (n=5) in the left panel and 2790±408 mg (n=5) in the right. Relaxations induced by $10^{-4}$ M papaverine were taken as 100% relaxation; mean absolute values in the strips treated with indomethacin and aspirin were 510±156 mg (n=5) and 414±141 mg (n=5), respectively. Significantly different from values with eperisone alone: $^{a}P<0.01$, $^{b}P<0.02$, $^{c}P<0.05$. Numbers in parentheses indicate the number of the preparations used. Vertical bars represent S.E.M.

eperisone were statistically insignificant. The activity of cyclooxygenase to produce PGH$_2$ from arachidonic acid was not influenced by eperisone in concentrations up to $10^{-3}$ M, but was inhibited by 89% at $10^{-6}$ M indomethacin. The IC50 value of indomethacin was approximately $2 \times 10^{-7}$ M.

Discussion

In dog saphenous artery and vein strips partially contracted with various agents, eperisone produced a concentration-dependent contraction or a relaxation. The effects depended on the agents that were used to
persistently contract the strips. Eperisone relaxed the saphenous artery and vein strips contracted with norepinephrine and serotonin. Treatment with eperisone attenuated contractions induced by norepinephrine and serotonin in the arteries, and it also reduced contractions due to clonidine and phenylephrine in the veins. Treatment of saphenous arteries with eperisone protected against the persistent blockade of α-receptors by phenoxylbenzamine. A similar protection of α-receptors was also observed with reversible, competitive antagonists (10, 14) and agonists (15). These results may indicate that eperisone blocks α₁ and α₂ adrenoceptors and serotonin receptors in vascular smooth muscle. Vascular serotonin (5-HT) receptor subtype is considered to be 5-HT₂ (16, 17), since ketanserin, a selective 5-HT₂ antagonist with some blocking activity, attenuates the serotonin-induced contractions in dog saphenous arteries and veins (present study), lowers systemic blood pressure and decreases peripheral vascular resistance (17).

Saphenous vein strips contracted with acetylcholine responded to eperisone with a relaxation. The relaxant response to acetylcholine of the artery strips, dependent on the endothelium, was suppressed by treatment with eperisone, whereas the relaxation caused by substance P was not affected in the indomethacin-treated strips. The peptide-induced relaxation was also endothelium-dependent. Therefore, it appears that eperisone does not interfere with the release and action of endothelium-derived relaxing factor (EDRF, ref. 18), but has an anti-muscarinic action. This action was observed in appreciably less concentrations, compared to blocking actions on α and 5-HT receptors (Figs. 3 and 4). The anti-muscarinic action of eperisone is also suggested by the fact that this drug antagonizes the specific receptor binding of ³H-quinuclidinyl benzilate in synaptic membranes prepared from the rat brain (19) and is effective in tremor of patients with Parkinson’s disease (20). Eperisone in a concentration of 10⁻⁴ M relaxed the vein strips contracted with K⁺ or Ba²⁺, but not the artery strips. These relaxing actions of eperisone may be explained by a possible involvement of non-specific vasodilatation action or Ca²⁺-entry blocking action (8). However, the reason for the difference between veins and arteries remains to be determined.

Transmural electrical stimulation produced a frequency-related contraction of saphenous artery strips. The arterial contraction and the ³H-overflow evoked by transmural stimulation in superfused saphenous artery strips previously soaked in the ³H-norepinephrine-containing medium were abolished by treatment with tetrodotoxin, suggesting that the induced contraction is derived from norepinephrine released from electrically stimulated adrenergic nerves. Prejunctional α₂ receptors play an important role in the negative feedback mechanism of the release of transmitter norepinephrine (21). Treatment with eperisone in a concentration of 10⁻⁵ M significantly increased the contractile response and the ³H-overflow as did yohimbine (22, 23). The potentiating effect of eperisone in the response to adrenergic nerve stimulation was attenuated by treatment with yohimbine in a concentration of 3×10⁻⁹ M, which would be sufficient to suppress the prejunctional α₂ receptor stimulation without a blockade of postjunctional α₂ receptors (22). These data together with those on blockade of postjunctional α₁ and α₂ receptors may indicate that prejunctional α₂ receptors play an important role in the release of norepinephrine upon nerve excitation, which overcomes the postjunctional α₁ and α₂ receptors blockade, resulting in a potentiation of the contractile response. Suppression by high concentrations (10⁻⁴ M or higher) of eperisone appears to be due to a greater blockade of postjunctional α₁ and α₂ receptors than that of prejunctional α₂ receptors.

Eperisone, like indomethacin and aspirin, produced concentration-dependent contractions in saphenous artery strips partially contracted with PGF₂α. Contractions caused by the cyclooxygenase inhibitors in PGF₂α-contracted arteries may be associated with reduction of PGI₂ synthesis stimulated by PGF₂α (24). Treatment with indomethacin or aspirin suppressed the eperisone-induced contractions. These findings led us to speculate that eperisone shares the action on cyclooxygenase activity with indomethacin and as-
pirin. However, the production of PGF$_{2\alpha}$ and 6-keto PGF$_{1\alpha}$ from arachidonic acid in saphenous arteries did not differ in the absence and presence of eperisone. Furthermore, the activity of cyclooxygenase to produce PGH$_2$ from arachidonic acid was not influenced by eperisone. Therefore, it is concluded that eperisone inhibits neither cyclooxygenase nor PGI$_2$ synthase. However, eperisone at $10^{-4}$ M significantly attenuated the relaxant response to angiotensin II, possibly mediated by PGI$_2$ (9), but did not influence the relaxation induced by exogenous PGI$_2$. These findings may indicate that the release of arachidonic acid is inhibited by eperisone, resulting in a decreased production of PGI$_2$ that participates in the contractions seen in the PGF$_{2\alpha}$-contracted arteries and veins (Fig. 8).

Eperisone is clinically used to relieve increased tone of skeletal muscle. The present study revealed a variety of actions of eperisone on the saphenous artery and vein, which regulate blood supply to the skeletal muscle in the lower limbs. The vascular actions so far obtained were as follows: (1) blocking actions on muscarinic, postjunctional a$_1$ and a$_2$ adrenergic, and serotoninergic receptors; (2) possible blockade of prejunctional a$_2$ receptors; (3) possible decrease in PG12 production; and (4) inability to reduce vasodilation mediated by EDRF and PG. These results indicate that the effects of eperisone are quite different from those of Ca$^{2+}$ antagonists and papaverine. Actions of eperisone on skeletal muscle blood flow may be determined by a balance of vasodilation (antagonism to postjunctional a$_1$ adrenergic and serotoninergic receptors) and vasoconstriction (antagonism to prejunctional a$_2$ receptors and decreased PG12 synthesis).

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