Identification of Capric Acid as a Potent Vasorelaxant of Human Basilar Arteries

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To determine whether naturally occurring fatty acids, especially saturated ones, might act directly as vasodilators, segments of human basilar arteries and umbilical arteries were precontracted submaximally with prostaglandin F\(_2\alpha\) and then exposed to different saturated fatty acids (C4 through C16) or unsaturated fatty acids (C14:1, C18:1, C18:2, and C18:3) at concentrations from 4 \(\mu\)M to 4 mM. The results showed caprate (C10) to be the most potent vasorelaxant and basilar arteries to be more responsive (EC\(_50\)=63 \(\mu\)M) than umbilical arteries (EC\(_50\)=780 \(\mu\)M). Caprate also inhibited contractions elicited by KCl, serotonin, and the thromboxane analogue U46619. The relaxation was independent of the endothelium, and potency was not related to the weak capacity of caprate to inhibit Ca\(^{2+}\)-induced contractions of K\(^+-\)depolarized basilar arteries. The pattern of potencies for the arteries differed, but among unsaturated fatty acids the monounsaturated (C14:1, C18:1) were more potent than the polyunsaturated (C18:2, C18:3). Comparing the potencies obtained with the concentrations reported for the free fatty acid content of arteries, brain, and plasma indicates that these lipids could influence vasomotion in health and disease. (Stroke 1991;22:469-476)

The diverse effects that saturated fatty acids have on enzymes suggest that these acids serve physiological functions beyond that of yielding energy. These acids, for instance, uncouple phosphorylation, inhibit Na,K-ATPase, inhibit adenylyl cyclase, and inhibit cytosolic guanylate cyclase but stimulate membranous guanylate cyclase.1-4 Moreover, each fatty acid may produce selective effects. Oleic acid more effectively inhibits Ca\(^{2+}\) influx into mast cells than other lipids and at physiological concentrations blocks 100% of an experimentally induced release of histamine.5 Among the saturated fatty acids, myristic acid was the most stimulatory of cyclic guanosine monophosphate (cGMP) production, and more so than several unsaturated ones.3 The reasons for such stereospecificity are unknown.

It is well known that the essential fatty acid arachidonate and its metabolites are vasoactive.6 However, isolated studies suggest that other fatty acids influence vasomotion. Thus, approximately 50% of the fatty acids found in arteries are derived from de novo synthesis from acetate,7 and high concentrations of free (nonesterified) fatty acids are stored in varying amounts in the intimal, medial, and adventitial layers, the concentrations averaging 1.8 mM.8 Also, these acids are metabolized differently from other tissues in that their metabolism does not require carnitine.9 Hyperlipidemia elevates the arterial free fatty acid content,8 and short-chain saturated fatty acids present in blood are found in the cerebrospinal fluid of animals10 and humans11 so that arteries not only manufacture nonesterified fatty acids but may be exposed to varying concentrations from other sources. The acids appear to be vasodilators as several short-chain fatty acids (C4 and C8) given intravenously were found to double cerebral blood flow in experimental animals without altering systemic blood pressure,12 and oleic acid was found to be twice as effective as arachidonate in inhibiting contractions of isolated bovine arteries.13 Although these latter findings indicate important vascular effects of saturated and unsaturated fatty acids, there has been no systematic study of the pharmacodynamic properties of fatty acids on isolated blood vessels.

Isolated arteries of laboratory animals may respond to vasoactive agents differently from the corresponding human vessels, and arteries from different regions may respond quite differently to the same agent.14-17 The present study was therefore performed primarily to determine whether fatty acids...
The umbilical arteries were precontracted with 10 mM KCl to 4 mM. The concentration was based on the report that 4 mM/kg of calcium produced prolonged, steady contractions. Once the tonic phase became evident (3-5 minutes), relaxation was allowed for 20 minutes until the maximal contractile response was obtained. The responses to KCl are independent of receptors, indicate the viability of the tissue, and correlate well with the responsiveness to other contractile agonists.

The basic protocol for this study was to precontract the arterial segment with an agent that in our experience produces prolonged, steady contractions. Once the tonic phase became evident (3-5 minutes), a fatty acid was applied cumulatively to the bath at concentrations of 4 μM to 4 mM. The 4 μM concentration was based on the report that 4 mM/kg of certain fatty acids given intravenously to cats selectively increases cerebral blood flow.

Prostaglandin F2α (PGF2α) and serotonin (5-HT) were used to precontract the arterial segments at concentrations estimated to represent EC50-90 from previous experience and by trial in each artery. The umbilical arteries were precontracted with 10 μM PGF2α or 1 μM 5-HT, and the basilar arteries were exposed to 6-10 (average 8.7) μM PGF2α. The thromboxane analogue U46619 was also used to precontract the basilar arteries. After washout of the drugs, a period of 30-40 minutes elapsed between experiments.

Similar studies were performed on precontracted arterial segments that had been reamed to destroy the endothelium, as previously described. In some experiments, the effect of fatty acids on the contractions elicited by CaCl2 in arterial segments exposed to calcium-free buffer and depolarized with KCl was determined. In addition, the effectiveness of the fatty acid to alter responses to CaCl2 and KCl was compared with that of the calcium channel blocker diltiazem.

The sodium salts of even-numbered saturated fatty acids from C4 to C16 (butyrate, caproate, caprylate, caprate, laurate, myristate, and palmitate) were studied for vasorelaxant properties. The unsaturated fatty acids studied were myristoleate (C14:1), oleate (C18:1), linoleate (C18:2), and linolenate (C18:3). The acids and PGF2α, 5-HT, caprylcarnitine, sodium β-hydroxybutyrate, sodium γ-aminobutyrate, and thrombin were purchased from the Sigma Chemical Co., St. Louis, Mo. Diltiazem was obtained gratis from Marion Laboratories, Inc., Kansas City, Mo. The stable thromboxane analogue U46619 (9,11-epithio-11,12-methano-TxA2) was provided by the Upjohn Co., Kalamazoo, Mich. Concentrated stock solutions of the experimental compounds were made so that only 10–100 μl stock solution was needed to achieve the final bath concentration of each substance. Fresh solutions were used, and only concentrated laurate and palmitate required heating to solubilize. The modest increase in bath pH produced by the fatty acids was unrelated to their potency as vasorelaxants, nor did the addition of NaHCO3 to increase the bath pH comparably have any vasorelaxant effect.

The results are expressed as mean±SEM grams or as percentage change from control. An appropriate Student's t test was applied to determine the level of significance. The EC50 values were determined from regression equations derived from computer analysis of the data.

Results

Figure 1 illustrates the basic protocol and shows that the saturated fatty acid caprate (C10) was clearly more potent at 0.4 mM than butyrate (C4), caproate (C6), or caprylate (C8) in relaxing basilar artery segments precontracted with PGF2α. However, C8 and C10 produced maximal relaxation, below the basal tone, at 4 mM while C4 and C6 lacked this property (Figure 1). The relaxant effect persisted unabated for at least 20 minutes and was completely reversed by washout of the fatty acids in that the contraction afterward to PGF2α was not diminished. There was no evidence of tachyphylaxis to the vasorelaxant effect (n=9 for C10 and n=7 for C8).
Among the saturated fatty acids, C10 was the most potent relaxant of the basilar artery (Figure 2). This was especially evident at 40 and 400 μM. The EC₉₀ for C10 was 63 μM in the basilar artery, seven times more potent than the next most potent saturated fatty acid (Table 1). C10 was also at least 19 times more potent than any of the unsaturated fatty acids, of which C14:1 and C18:1 were the most potent and were equieffective (Table 1).

The basilar artery was more responsive to C10 than the umbilical artery, with EDₕ₀s of 63 μM and 780 μM, respectively (Table 1). The notable differences in the potency of C10 are summarized in the concentration–response curves of Figure 3. The unsaturated fatty acids were less potent than C10 in both types of vessels (Table 1). In the basilar artery, however, the monounsaturated fatty acids were more potent than the polyunsaturated, whereas in the umbilical artery the relation between a double bond and potency was not as evident (Table 1). Nevertheless, the most abundant mammalian fatty acid, oleate (C18:1), was more potent in the basilar artery than in the umbilical artery and more potent than some of the saturated fatty acids.

To ascertain whether the saturated fatty acids act synergistically, six basilar artery segments were precontracted with PGF₂α and the effects of the EC₉₀ for C8 and C10 (Table 1) were measured separately and in combination. The relaxant response to C8 alone averaged 49±9.2%, that to C10 46±8.3%, and that to the combination 90.7±5.6%. The results indicate an additive effect, being within 10 percentage points of the expected combined effect. Also, at random intervals 0.4 mM C10 was applied to vessel segments that had failed to relax completely in response to other fatty acids, and the first acid never interfered with the response to C10.

U46619 was used to precontract six basilar artery segments from three individuals to determine whether a different contractile agent influenced the response to C10 (data not shown). The vessels were alternately contracted with the EC₉₀ for PGF₂α and equieffective concentrations of U46619, usually 10 or 100 nM. The agonists were applied twice to each segment, and the contraction elicited by PGF₂α averaged 12.5±0.3 g and that elicited by U46619 12.1±1.4 g. The relaxant effects that 4 μM to 4 mM C10 had on the contractions generated by each agent did not differ significantly (Student’s t test for paired data), the maxima being 105.1% after U46619 and 107.6% after PGF₂α.

Among the other compounds tested, caprylcarnitine at 1 μM was nearly as effective as C10 in inhibiting the contractions produced by 1 μM 5-HT in the same umbilical artery segments, reducing the contractions by 49.9±12.3% and 67.7±5.8%, respectively (n=7) (data not shown). While the inhibitions were not significantly different (Student’s t test for paired data), caprylcarnitine was on average less effective and less predictable as reflected in the SEM. In four basilar artery segments precontracted with PGF₂α the application of β-hydroxybutyrate at 0.4 mM had no effect and at 4 mM reduced the contraction by only 20.6±1.8%. At comparable concentrations γ-aminobutyrate relaxed the vessel.
The profile of potency for the saturated fatty acids also differed, resembling a distorted V for the basilar artery (Figure 2, Table 1) while being more L-shaped for the umbilical artery (Table 1). Thus, in basilar arteries potency decreased markedly as carbon length changed from C10, whereas potency changed little in the umbilical arteries as carbon length increased from C10. Also, differences between the responses to C10 and C8 or C12 were significant in the basilar artery \((p<0.05)\) but not in the umbilical artery.

Contractions elicited in umbilical artery segments by 40 mM KCl, due to an influx of \(\text{Ca}^{2+}\) through voltage-operated channels, were inhibited only when C10 or diltiazem was administered 6–8 minutes before KCl (Figure 4). By extrapolation, the calcium antagonist was about 500 times more potent than C10. The contractions produced by 5-HT, which opens receptor-operated calcium channels, were affected differently by diltiazem and C10 in that inhibition occurred whether the agents were administered before or after 5-HT (Figure 4). An estimate of the EC_{50} indicated that diltiazem was only about 66 times more potent than C10 against 5-HT-induced contractions. In any case, diltiazem and C10 pro-

\[ \text{by } 6.5\pm2.1\% \text{ and } 13.6\pm2.8\%, \text{ respectively, while C10 diminished the contraction by } 86.7\pm1.3\% \text{ and } 104.3\pm0.8\%, \text{ respectively.} \]
duced qualitatively similar effects on the contractions elicited by KCl and 5-HT.

Figure 5 illustrates the protocol and one result of experiments designed to better define the effect C10 has on transmembrane Ca\(^{2+}\) exchange. The artery segments were first exposed to calcium-free buffer for approximately 2 hours, then depolarized with 40 mM KCl; 3–5 minutes later 0.1–10 mM CaCl\(_2\) was added to elicit contractions (tracing 1, Figure 5). After five or six washouts with calcium-free buffer and an elapsed time of about 60 minutes, the experiment was repeated but with 0.4–4 mM C10 added to the bath 6–8 minutes prior to K\(^+\) depolarization. The contractions elicited by high concentrations of CaCl\(_2\) in the basilar artery were unaffected by 0.4 mM C10 but were inhibited significantly by 4 mM C10 (Figure 6). In contrast, all concentrations of C10 inhibited the Ca\(^{2+}\)-induced contractions in the umbilical artery, and at 4 mM the response was abolished (Figure 7). Diltiazem segments were first exposed to calcium-free buffer for approximately 2 hours, then depolarized with 40 mM KCl; 3–5 minutes later 0.1–10 mM CaCl\(_2\) was added to elicit contractions (tracing 1, Figure 5). After five or six washouts with calcium-free buffer and an elapsed time of about 60 minutes, the experiment was repeated but with 0.4–4 mM C10 added to the bath 6–8 minutes prior to K\(^+\) depolarization. The contractions elicited by high concentrations of CaCl\(_2\) in the basilar artery were unaffected by 0.4 mM C10 but were inhibited significantly by 4 mM C10 (Figure 6). In contrast, all concentrations of C10 inhibited the Ca\(^{2+}\)-induced contractions in the umbilical artery, and at 4 mM the response was abolished (Figure 7). Diltiazem...
The remarkable sensitivity manifested by the basilar artery supports the hypothesis of others that fatty acids participate in the normal regulation of cerebral blood flow. It is possible, however, that unknown postmortem changes contributed to the greater sensitivity since the umbilical arteries were in general prepared for use sooner than the basilar arteries. On the other hand, the responses to well-known contractile agonists of “fresh” basilar arteries and human basilar arteries prepared for use 7–24 hours postmortem are similar, and there is little change in vasomotor activity as assessed by absolute contraction or molar sensitivity during the first 24 hours after death. Likewise, the sensitivity to several agonists of “fresh” canine and postmortem human basilar arteries is remarkably similar; the sensitivity to 5-HT is identical. The literature also indicates that “fresh” human pial arterioles removed during surgery and larger cerebral arteries removed postmortem respond in a similar manner to most agonists. In addition, we have noted in preliminary reports that canine basilar arteries are as sensitive to C10 as human basilar arteries (EC50 of 49 μM and 63 μM, respectively) and more sensitive than canine femoral arteries (EC50 of 440 μM). Together these findings suggest that differences in sensitivity to C10 were not due to the “freshness” of the vessel nor to its size and that among the vessels studied cerebral arteries are the most sensitive, independent of species.

The mechanism involved in the efficacy of C10 as a dilator remains problematic. The endothelium was not a requirement as it is for many dilator agents. Its potency as an inhibitor of PGE2-induced contractions in umbilical arteries and as an inhibitor of Ca2+-induced contractions suggests that C10 was producing calcium blockade in that artery. In the basilar artery, however, there was no such relation, with 400 μM C10 having no effect on Ca2+-induced contractions. Although palmitate (C16) and palmitoylcarnitine have diametrically opposite effects on Ca2+-induced responses of intestinal strips, both C10 and its carnitine ester were vasorelaxant in basilar artery segments.

Membranous guanylate cyclase of human fibroblasts is one enzyme that is directly stimulated by either saturated or unsaturated fatty acids at physiological concentrations, 100–600 μM. This stimulation is greatest in the presence of Mg2+, a divalent cation that favors vasorelaxation. In contrast, nitrovasodilators do not stimulate the enzyme, which may explain why fatty acids relax the umbilical artery (Table 1) whereas sodium nitrite does not. Although the vasorelaxation might be mediated by cGMP, the investigation of arteries is required because several saturated fatty acids, especially C14, stimulate the enzyme more effectively than does C10. Also, unsaturated fatty acids are nearly as effective as C14 in the generation of cGMP, whereas C10 was far more vasorelaxant in the basilar artery (Table 1). Nevertheless, it is of special interest that these findings clearly demonstrate that fatty acids are vasorelaxants, that potency varies with carbon length and saturation, that caprate (C10) is the most potent, and that basilar arteries are more sensitive to C10 than are umbilical arteries, with EC50s of 63 μM and 780 μM, respectively. Moreover, C10 was antagonistic to a variety of contractile agonists. Although less potent, two of four unsaturated fatty acids, myristoleate (C14:1) and oleate (C18:1), relaxed precontracted basilar arteries at EC50s of approximately 1 mM (Table 1). Since the dilation produced by each fatty acid was prolonged, the nonesterified fatty acids normally synthesized and stored by arteries could exert a tonic relaxant effect on blood vessels.
fatty acids manifest a stereospecificity for the enzyme and as vasorelaxants.

The stereospecificity manifested by fatty acids in other tissues also seems unrelated to the vasodilator action. For instance, C14 is the most potent uncoupler of oxidative phosphorylation, and C18:1 proved to be the best inhibitor of histamine release by mast cells. Also, attempts to link the hypnotic effect of saturated fatty acids to specific enzymes have been unsuccessful. Comparative studies show, however, that C8 and C10 are more potent in producing dose-dependent hypnosis and coma in animals than are C4 or C6. This ranking roughly parallels the vasodilator potency pattern seen in arteries (Figure 1). On the other hand, C4 was not a potent dilator of human basilar arteries (Table 1) but reliably produces sleep and increases cerebral blood flow in cats. The difference may be related to a central nervous system effect, a species effect, and/or a greater sensitivity of arterioles to fatty acids. Although the hypnotic effect might limit the clinical use of C10 as a vasodilator, in our experience femoral blood flow of dogs can be doubled by the intraarterial injection of C10 without systemic effects or deepening anesthesia.

The concept that nonesterified fatty acids influence vasomotion is further supported by the concentrations revealed in isolated studies. Thus, a group of cellular low-molecular-weight proteins bind up to 500 \( \mu M \) of nonesterified fatty acid and one binds only saturated fatty acid. Feline serum normally contains 180 \( \mu M \) C4, 10 human serum 2–18 \( \mu M \) C8, and rabbit brain 200 \( \mu M \) C8. The concentration of nonesterified fatty acids in canine aortas varies with the layer studied, from 660 \( \mu M \) to 2.9 \( \mu M \), while 680 \( \mu M \) is normally present in serum. A fatty diet including butter (30% C4–C10) markedly increases the arterial fatty acid content, especially in the muscle layer. Also, sympathetic nerves of rats fed saturated fats (C8–C18) store far more and release much less norepinephrine than rats fed other diets. The tail arteries of rats fed saturated fats also respond on average less to norepinephrine. With ischemia, the brain concentration of saturated fatty acids exceeds by twofold that of arachidonate and in one animal reached 1.2 mM. Electroconvulsive shock produces a similar free fatty acid profile, and these acids are derived from phospholipids, not triglycerides. In Reye's syndrome plasma levels of 11.5 mM (170 mg/dl) have been reported for octanoate (C8), and the concentration of this fatty acid best reflected the clinical condition of the patient, including coma. Also, alimentation of C8 to patients may yield serum values of 1.1 mM, will elevate the cerebrospinal fluid concentrations, and may produce coma. Medium-chain (C8) and long-chain (C14) fatty acids are actively transported through the blood-brain barrier so that the brain, plasma, and cerebrospinal fluid are sources of saturated fatty acids that, under pathological conditions, could alter cerebrovascular tone. Our findings support the posited that the increases in intracranial pressure observed in patients with Reye's syndrome could be due to cerebrovasodilation caused by elevated fatty acid levels.

The dilator response may also reflect metabolic states as fat depots with the highest lipid turnover rates have the highest blood flows. The type of fatty acid stored might also account for the fact that many obese individuals are normotensive. Conversely, since arteries in diabetic patients do not synthesize fatty acids but do synthesize cholesterol, the poor circulation often seen in such patients may reflect this deficiency. In any case, the comparison of reported concentrations present in arteries and other tissues with their potencies as vasodilators indicates that fatty acids could play a role in vasomotion in health and disease.

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