Histamine-Induced Rhythmic Contraction of Hog Carotid Artery Smooth Muscle

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SUMMARY. Smooth muscle strips isolated from the hog common carotid artery can contract rhythmically, exhibiting low frequency, large amplitude oscillations in tension when stimulated with 10 μM histamine. Strips required at least 1.45 mM calcium and 2.5 mM potassium to exhibit this rhythmic activity. Rhythmic contractions could be converted to tonic contractions by removal of potassium or ouabain treatment. The involvement of adrenergic nerve terminals in this response was ruled out, since propranolol, phentolamine, tetrodotoxin, bretylium, or 6-hydroxydopamine treatment did not alter the oscillations. Blockade of H1 receptors with 0.1 μM diphenhydramine relaxed the muscle strips. The H2 receptor antagonist cimetidine (5 μM) had no effects. Attempts to obtain rhythmic contractions by stimulating with other vasoactive agents (norepinephrine, acetylcholine, 5-hydroxytryptamine, angiotensin II, and elevated potassium concentrations) were unsuccessful, suggesting that this is a specific histamine response mediated solely by H1 receptors. These results show that this large artery, commonly considered a multi-unit smooth muscle, can sometimes exhibit single-unit behavior. (Circ Res 55: 480-485, 1984)

SMOOTH muscle is often classified according to its functional characteristics as either single-unit or multi-unit (Bozler, 1941). Large arteries, including the hog carotid, are considered to be good examples of multi-unit smooth muscle. This type of smooth muscle is characterized by the lack of myogenic and rhythmic activity, the presence of very few gap junctions, the inability to propagate electrical activity, and the ability to respond to excitatory stimuli with only graded, tonic contractions (Somlyo and Somlyo, 1968a, 1968b). Recently we found that histamine stimulation can make the hog carotid artery contract rhythmically, an ability that apparently conflicts with the multi-unit classification. This is interesting because the effect is dose-dependent; at high concentrations, histamine contractions are tonic, as expected for a multi-unit muscle.

There are some reports of rhythmic contraction of other multi-unit smooth muscles in the recent literature. Only a few of these reports describe regular, large amplitude rhythmic contractions in most of the tissues studied (Biamino and Kruckenberg, 1969; Ross et al., 1980; Ginsburg et al., 1980; Bose and Bose, 1977). Many of these instances of rhythmic contraction may result from pathological states (Bandick and Sparks, 1970; Bohr and Sirrin, 1970; Ginsburg et al., 1980; Ross et al., 1980; Golenhofen et al., 1981; Ueda et al., 1981) or deliberately nonphysiological conditions (Bose and Bose, 1977; Kannan and Daniel, 1978). Also, in some of these studies, the rhythmic component of force was small and superimposed on a much stronger tonic contraction (Johansson and Bohr, 1966; Bevan and Ljung, 1974) or the rhythmic contractions were noted only occasionally (Barr et al., 1962; Norton and Detar, 1972) or during equilibration at 37°C after cold storage (Barr et al., 1962).

In contrast, the rhythmic contractions we report here are strong, with only a small tonic component of force; they are demonstrable in a large fraction (75%) of the arteries tested; they occur with tissue from normal animals; and they result from stimulation by a physiologically occurring agonist. The simple conditions required to elicit this response, its reproducibility, and the large amplitude of the contractions, suggest that this may be a fundamental property of large artery smooth muscle that has received little attention. The experiments reported here were undertaken to characterize this response.

Methods

Common carotid arteries were collected from 100- to 400-kg swine of either sex, at slaughter. Any thrombi in the vessels were rinsed out, and the arteries were placed in 0°C physiological saline solution (PSS) of the following composition (in mM): NaCl, 117.8; NaH2PO4, 1.2; Na2EDTA, 0.027; KCl, 6.0; CaCl2, 1.6; MgSO4, 1.2; NaHCO3, 24.3; and glucose, 5.6. Gassed with 95% O2, 5% CO2, this PSS has a pH of 7.4 at 37°C. Arteries stored in this PSS at 4°C remain viable for 2–3 days. The minimum time from the death of the animals to mounting of a strip in the muscle bath was about 3 hours.

Medial-intimal strips were prepared as described pre-
viously (Driska et al., 1981). Strips were blotted for approximately 10 seconds on absorbent paper and weighed before the experiments. Strips which were not blotted and weighed were also capable of contracting rhythmically. Strips 2–3 mm wide were mounted with stainless steel clips to an apparatus which allowed strip length to be measured and changed with a resolution of 0.1 mm. Force was measured with Grass FT.03C transducers and recorded on Grass or Beckman recorders. The total mechanical compliance of this apparatus was 5 mm/g. Strips were immersed in 50 ml of 37°C PSS which was gassed continuously with 95% O₂, 5% CO₂. Strips were then stretched in one motion to a length yielding 10 g tension and allowed to equilibrate for at least 1 hour or until tension returned to baseline.

Dose-Response Curves

Normalized dose-response curves for tonic active force in response to histamine were constructed and the data for six tissues averaged. The ED₅₀ value was determined from the mean data by linear regression analysis using the equation log₁₀(F/Fₐ₀-F) = log₁₀D - log₁₀ED₅₀. Here, F represents the force elicited by a given dose (D) of histamine, and Fₐ₀ represents the maximum force obtained (with 30 μM histamine). The slope of the fitted line was 1.85, the correlation coefficient was 0.995, and the ED₅₀ was 3.4 μM. Dose-response curves in the presence of 20 μM cimetidine were identical to those obtained in its absence.

Solutions

Length-tension relationships were determined (Herlihy and Murphy, 1973), using K⁺ PSS stimulation. The K⁺ PSS had the same composition as the PSS described above, except for an equimolar substitution of KCl for NaCl. Experiments involving La³⁺ and Mn²⁺ required the use of a saline solution of the following composition (in mM): NaCl, 140; MgSO₄, 1.2; CaCl₂, 1.6; KCl, 6; D-glucose, 5.6; and N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 5.0 Gassed with 100% O₂, this PSS had a pH of 7.45 at 37°C. Histamine dihydrochloride (HA), norepinephrine (NE), 6-hydroxydopamine, tetrodotoxin (TTX), ouabain, acetylcholine (ACh), and 5-hydroxytryptamine (5-HT) were purchased from Sigma. Tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) were purchased from Aldrich. Verapamil was a gift of Knoll Laboratories, and Smith, Kline, and French.

Statistical Analysis

Means, ranges, and standard deviations were used to express the variability of the oscillations in different strips with respect to latency, amplitude, and frequency. In a preliminary report (Stein and Driska, 1981), 128 (75%) of the 172 strips tested showed oscillations; since that time, the total number of strips has increased to several hundred with about the same percentage showing oscillations. In studies of agents which inhibited rhythmic contractions, inhibition was defined as a decrease of over 90% in the amplitude of the force oscillations. Because strips sometimes stopped contracting rhythmically and relaxed spontaneously, it was important to estimate the probability that the oscillations stopped by coincidence rather than as a result of the intervention. In the data presented for inhibitory agents, the number of strips tested (n) is given, and each test had a successful result. The probabilities of these results being coincidences were computed using the binomial distribution, assuming equal chances of a result being due to coincidence or being due to the intervention. Thus, the probability of the results due to chance is (½)ⁿ, or 0.125 for n = 3, 0.0625 for n = 4, 0.031 for n = 5, 0.016 for n = 6, and 0.008 for n = 7. In experiments in which no effect of an agent was found, the number of experiments, n, was between 3 and 6, unless otherwise noted.

Results

Mechanical Characteristics of the Contractions

When hog carotid artery smooth muscle strips are stimulated with 10 μM HA, most (75%) exhibit the response seen in Figure 1. The general response is a tonic contraction of variable duration (2 to 30 minutes, mean = 14 min) which then develops rhythmicity, i.e., oscillations in force. Fifteen to 30 minutes after the onset of oscillations, the force excursions reach a maximum with a fairly constant amplitude and frequency (range: 7.5 to 43/hr, or periods of 1.4–8 minutes). Whereas the frequency is fairly constant for a given tissue, there is much variation between tissues.

The rhythmic component of contraction is strong; it is not simply a small tension fluctuation superimposed on a strong tonic contraction. In a random sample of 25 of the arteries studied, the peak rhythmic force was 116.0 ± 51% (SD) of the tonic force produced by histamine. The force decreased to 36.9 ± 11% (SD) of the tonic histamine force (or 32% of the peak force) during the relaxation phase, although in some cases relaxation was more complete (<10% of tonic force). Strips continued contracting in this fashion for up to 6 hours. When rhythmic activity stopped, most strips began to relax progressively less between force peaks, eventually developing a tonic contraction.

Histamine concentrations between 5 and 10 μM routinely produced rhythmic contractions. Occasionally, concentrations as low as 8 × 10⁻⁷ M and as high as 4 × 10⁻⁵ M produced oscillations. Concentrations that were too high produced strong tonic contractions. Dose-response curves of tonic contractile force with HA stimulation (not shown) have an
ED$_{50}$ of 3.4 mM, and 10 µM histamine elicits 88% of the maximum tonic force. Since rhythmic contractions did not usually occur at higher HA concentrations, it seems that full occupancy of histamine receptors is incompatible with rhythmic activity.

Strips were routinely stretched to reach 10 g force when first mounted in the apparatus, and experiments were done at the length determined by this stretch. Length-tension curves constructed at the end of some experiments determined that the 10 g stretch resulted in a strip length of 0.63 ± 0.04 $L_o$, where $L_o$ is the optimal length for force development. At 0.63 $L_o$, active stress with 10 µM HA stimulation is 1.39 ± 0.24 × 10$^5$ N/m$^2$ (sd, n = 25), which is about the same as that with K$^+$ PSS.

Arteries were routinely transported in 0°C PSS, but on one occasion we collected and transported arteries to the laboratory in warm (29–33°C) PSS. Two muscle strips from each of six arteries were studied; of these 12 strips, six contracted rhythmically. This shows that rhythmicity was not a consequence of cold storage of the arteries.

Involvement of Nerve Terminals

It has been reported that the swine carotid artery cannot propagate electrical activity (Burnstock and Prosper, 1960). An alternate mechanism for synchronizing contractions in a tissue incapable of propagated electrical activity would be through synchronized release of norepinephrine from the network of adrenergic nerve endings in the preparation. Several experiments designed to test for such a mechanism failed to produce evidence for nerve terminal involvement.

Tetrodotoxin (TTX), by blocking fast Na$^+$ channels in adrenergic nerves, should block both conduction along a nerve network and release of NE from nerve terminals mediated by action potentials. However, 1 µM TTX did not affect the rhythmic contractile activity. Neither 10 µM bretynil tymosylate (a blocker of norepinephrine release) nor 10 µM phenolamine (an $\alpha$-adrenergic receptor blocker) had any effects on the force oscillations. $\beta$-propranolol (10 µM) also had no effect, which rules out the possibility of $\beta$-receptors mediating the relaxation phase of the rhythmic contractions. Finally, treatment of the strips with 6-hydroxydopamine to destroy adrenergic nerve terminals (Aprigliano and Hermsmeyer, 1976) did not prevent rhythmic contractions when strips were later stimulated with histamine (n = 7). These experiments rule out rhythmic synchronized NE release from nerve terminals as the cause of the rhythmicity of the contractions.

Prostaglandins

Prostaglandins have recently been implicated in the rhythmic contraction of human renal artery strips (Ueda et al., 1981). We routinely blotted the muscle strips with filter paper for weighing before the experiments, and this destroys the endothelial cells which are likely to be the site of prostaglandin generation. Exposure of the strips to 80 µM indomethacin (a prostaglandin synthesis inhibitor) for 20 minutes prior to histamine stimulation had no effect on the oscillations, nor did indomethacin when it was added during the oscillations (n = 3). These facts suggest that prostaglandin formation is not involved in the rhythmicity.

Histamine Receptor Studies

Both H$_1$- and H$_2$-histamine receptors are present in the vasculature, and one possible explanation for the oscillations was that one type of histamine receptor was responsible for the contraction and the other for the relaxation. The addition of 0.1 µM diphenhydramine, an H$_2$ antagonist, caused rapid and complete relaxation, as shown in Figure 2 (n = 5). When 10 µM HA was replaced by the H$_1$ agonist, 2-methylhistamine (10 µM), the rhythmic contractions were unchanged (n = 4), confirming that H$_1$ receptors are responsible for the oscillations. Cimetidine (5 µM), an H$_2$ antagonist, had no effect. Addition of 10 µM 4-methylhistamine (an H$_3$ specific agonist) to strips stimulated by either HA or 2-methylhistamine had no effect. As expected, 4-methylhistamine alone did not cause the strips to contract (n = 4). These results show that activation through H$_1$ receptors alone is sufficient to generate rhythmic activity, and that H$_2$ receptors do not have a role in the response.

Ionic Requirements

The Ca$^{2+}$ concentration in the bathing medium has a marked effect on oscillatory activity. Addition of 1.6 mM EGTA to the normal PSS (1.6 mM Ca$^{2+}$) or substitution of the normal PSS with one nominally free of Ca$^{2+}$ stopped the oscillations (n = 5). CaCl$_2$ was added back to the tissue baths in 0.2 mM increments to determine the [Ca$^{2+}$] threshold for resumption of oscillations, which was 1.45 mM ± 0.18 (sd, n = 8).

![Figure 2. Relaxation by histamine H$_1$ receptor blockade. Diphenhydramine was added to give a bath concentration of 10$^{-5}$ M. The time scale is aligned so that zero corresponds to the time that diphenhydramine was added. The histamine concentration was 10$^{-5}$ M throughout.](http://circres.ahajournals.org/Download)
Transmembrane Ca++ flux appears to be necessary for oscillations. Blockade of the slow channels with 2 mM La+++ (n = 7), 1 mM Mn+++ (n = 6), or 1 mM verapamil (n = 4) (all in the presence of 1.6 mM CaCl2) rapidly stopped the oscillations and relaxed the smooth muscle. The ability of verapamil to block the oscillations suggests that it is the entry of Ca++ into the cell (rather than the binding of Ca++ to sites in the tissue or "stabilization" of the membrane) that is required for the rhythmic contractions.

Potassium was also required for the rhythmic response. If muscle strips were incubated in K+-free PSS for 20 minutes, HA produced only tonic contractions on stimulation (n = 20). When rhythmically contracting strips in normal PSS were switched to a K+-free PSS containing 10 μM HA, the rhythmic contractions were converted to a tonic contraction. Rhythmicity could be restored by the reintroduction of K+ to the bath (n = 7), as shown in Figure 3. When K+ was introduced in 0.2 mM increments, oscillations reappeared when K+ reached a mean value of 2.5 ± 0.62 mM (sd, n = 7). Elevation of [K+] to 30 mM produced sustained contractures.

Ouabain

Ouabain (1 μM) rapidly converted the rhythmic contractions of the strips to sustained contractures (Fig. 4, n = 4). This shows that Na-K pump activity is required either in a direct way as a pacemaker or indirectly in maintaining membrane potential and/or concentration gradients in specific ranges.

Other Vasoactive Agents

To determine whether the rhythmic contractions were specific for histamine, we attempted to elicit rhythmic contractions using the following vasoac-

They oscillations to a tonic contraction. Additions of KCl to give increments are denoted by each successive arrow. Bath K* concentration is shown above the force record.

**Discussion**

It is appropriate to consider possibilities which could artifactually cause the behavior reported here. Some of these possibilities are listed below. Arteries were routinely flushed with cold PSS at the slaughtered house to remove blood or thrombi. Rhythmic contractions were also exhibited in experiments in which only arteries free of thrombi were collected, so it did not appear that the oscillations were due to prior exposure of the blood vessels to thrombi. Cooling arteries to 0°C in PSS for transport, dissection, and storage is a common procedure, but it raises questions of possible alterations in function, especially with regard to receptors and the integrity of nerve endings in the preparation. Cold storage also causes loss of K+ from cells and accumulation of Na+, a situation that results in strongly electrogenic Na+ extrusion on warming. Such a mechanism was suggested for the rhythmicity occasionally observed in dog carotid artery strips warming after cold stor-
age (Barr et al., 1962). We do not think that prior cold storage is the explanation for the oscillations reported here for the following reasons: (1) strips were allowed to equilibrate for 2 hours at 37°C before stimulation with HA; (2) histamine also caused rhythmic contractions in strips which had been stimulated repeatedly with K⁺ PSS over a period of hours, showing that rhythmicity is not an artifact restricted to the first contraction at 37°C; and (3) strips from arteries that were collected and transported to the laboratory in warm PSS also contracted rhythmically in 10 μM HA.

The rhythmic mechanical activity implies that there must be both a pacemaker and a means of coordinating the responses of the individual cells. By using blockers of NE release, blockers of adrenergic receptors and, finally, 6-hydroxydopamine treatment to destroy the nerve terminals, we have shown that the adrenergic nerve terminals cannot be the pacemaker or the means for coordinating the muscle cell response. This suggests that smooth muscle cells are the source of the rhythmicity. These results also imply some mechanism for coordinating cells in the tissue, yet this conflicts with the finding that there is no conduction in the hog carotid (Burnstock and Prosser, 1960). One explanation for this apparent conflict is that the carotid artery might conduct electrical activity only when exposed to histamine or other contractile agonists.

Histamine stimulation of arterial smooth muscle usually causes an initial rapid phase of contraction thought to correspond to the release of Ca²⁺ from internal stores, and a slower tonic phase thought to reflect the entry of Ca²⁺ from the extracellular space (Hudgins and Weiss, 1968; Watkins and Davidson, 1980). The sources and sinks for the Ca²⁺ ions involved in the rhythmic contractions are not known, but the experiments with La³⁺, Mn³⁺ and verapamil indicate that influx of extracellular Ca²⁺ is required. Histamine has been shown to increase Ca²⁺ permeability in other arterial smooth muscles (Harder, 1980). However, this does not rule out participation by intracellular Ca²⁺ pools, e.g., Ca²⁺-induced Ca²⁺ release from the sarcoplasmic reticulum. The necessity of extracellular calcium for the rhythmic contractions suggests that the contraction amplitude would depend on [Ca²⁺]. However, a simple monotonic relationship between peak force and [Ca²⁺] was not found (unpublished results).

Histamine-induced contractions did not show rhythmicity when the K⁺ concentration of the bathing solution, [K⁺]b, was less than 1.2 mm. Potassium-free solutions usually depolarize vascular smooth muscle, whether through inhibition of an electrogenic Na-K pump, a decrease in potassium conductance, or indirectly, through norepinephrine release from nerve endings (for a review, see Hermansmyer, 1983). Such a depolarization might prevent rhythmicity. The [K⁺]b requirement for rhythmicity could also arise through its role in Na-K pump activation or through mechanisms involving rhythmic conductance changes. The most likely interpretation of this K⁺ requirement is that the electrogenic Na-K pump in the smooth muscle membrane must be operating for the rhythmic behavior to develop. Relaxation of vascular smooth muscle following the reintroduction of K⁺ to a K⁺-deficient bathing medium is thought, by some authors, to be due solely to the stimulation of the electrogenic Na-K pump and the concomitant hyperpolarization of the membrane (Biamino and Wessel, 1973; Hendrickx and Casteeles, 1974; Webb and Bohr, 1978; Hermansmyer, 1983). The fact that ouabain rapidly stops the oscillations and causes a tonic contracture is another indicator that the activity of the Na⁺,K⁺-ATPase is a necessary component of the relaxation phase of this phenomenon. The effect of ouabain is rapid enough in these experiments (less than 1 minute in Fig. 4) that it probably is due to a loss of an electrogenic component of membrane potential, rather than to other consequences of ouabain treatment, such as Na⁺ accumulation or K⁺ depletion.

Rhythmic contractions are often considered an unusual response for a multi-unit tissue like the hog carotid artery. However, there have been reports of similar behavior in other multi-unit tissues in the recent literature (see introduction), and in the early 1900’s there were a few reports of large arteries showing single-unit properties like rhythmic contraction and myogenic responses. These results came from several arterial tissues; dog carotid (Bayliss, 1902), cow renal and mesenteric (Rothlin, 1920), horse carotid (Wachholder, 1921), and cow carotid (Full, 1913; Müller, 1906). We do not mean to suggest that the hog carotid artery is really a single-unit smooth muscle, nor do we advocate discarding the single-unit/multi-unit classification system. The major point we wish to emphasize is that, under certain experimental conditions, multi-unit tissues like the hog carotid artery can exhibit single-unit behavior. It is not known whether this unusual behavior is related to the rhythmic contractions sometimes seen in pathological arteries. Even though it is probably an oversimplification, the classification of smooth muscle as either single-unit or multi-unit has been very useful in our understanding of smooth muscle physiology over the years. With the understanding that such a classification might not be true under all conditions, this concept should still provide a useful framework for smooth muscle research in the future.

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