Abnormal Response of the Pulmonary Artery of the Rabbit after High Frequency Sympathetic Nerve Stimulation

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ABSTRACT

When the sympathetic nerve supply (right recurrent cardiac nerve) to a ring of the pulmonary artery of the rabbit is stimulated repetitively in vitro at frequencies of 10/second or more, the following changes occur which are not seen after stimulation at lower frequencies. (1) The minimum number of pulses in a train needed to cause a just-detectable contractile response is reduced from a mean of 7 to unity; the higher the frequency of repetitive stimulation the fewer the number of repetitive pulses needed to effect this change. (2) The basal tone of the blood vessel exhibits fairly rapid, small, spontaneous fluctuations. (3) The resting or basal tone of the vessel slowly increases for ½ to 2 hours. It is speculated that changes 1 and 2 may be the result of an irreversible change in transmitter storage or release mechanisms.

ADDITIONAL KEY WORDS

spontaneous changes in vascular tone
adrenergic transmitter
\(\alpha\)-adrenergic blocking agents
arterial innervation
facilitated transmitter release
increased vascular tone

Recent studies with the isolated sympathetic nerve-pulmonary artery preparation of the rabbit (1) have demonstrated that the sympathetic nerve supply to this blood vessel must be stimulated with a train of 5 to 9 pulses before a contractile response is detected (2). Although the precise number of pulses to cause a just-detectable response varies from one preparation to another, it is constant in any one preparation and independent of pulse frequency between 5 and 50/second.

In the course of some pilot studies designed to elucidate factors that might modify the minimum effective number of pulses, we found that prior stimulation of the sympathetic nerve with frequencies of 25/second irreversibly reduced the minimum effective number of pulses to unity. After 2000 pulses delivered at a frequency of 25/second, the response to a single pulse was equal to that following a train of 10 to 15 pulses before this prolonged stimulation. Coincident with this change, the resting or basal tone of the blood vessel increased slowly for ½ to 2 hours to a new level, and, in addition, the artery began to show rapid, spontaneous changes in tone. None of these changes were seen when its nerve was stimulated with the same number of pulses at a frequency lower than 10/second.

Evidence will be presented which suggests that the reduction in the minimum effective number of pulses to unity and the spontaneous, irregular changes in tone may be the consequence of an irreversible and deleterious change in the mechanisms that govern the release and storage of the adrenergic transmitter in the sympathetic nerve terminals in the artery.

Methods

The apparatus and the general experimental procedures were similar to those described before (1, 2). The sympathetic nerve-pulmonary artery preparation of the rabbit consisted of the ring formed by the distal 5 to 7 mm of the main
Diagram of experimental arrangement of a 5- to 7-mm segment of the main pulmonary artery and attached right recurrent cardiac nerve of the rabbit. The tissue and electrodes are immersed in modified Krebs bicarbonate saline solution equilibrated with 95% O₂-5% CO₂ at 38°C.

Diagram of experimental arrangement of a 5- to 7-mm segment of the main pulmonary artery and attached right recurrent cardiac nerve of the rabbit. The tissue and electrodes are immersed in modified Krebs bicarbonate saline solution equilibrated with 95% O₂-5% CO₂ at 38°C.

Diagram of experimental arrangement of a 5- to 7-mm segment of the main pulmonary artery and attached right recurrent cardiac nerve of the rabbit. The tissue and electrodes are immersed in modified Krebs bicarbonate saline solution equilibrated with 95% O₂-5% CO₂ at 38°C.
was determined for each preparation; this varied between 10 and 30 volts. Thereafter, a supramaximum voltage (5 to 10 volts greater than just-maximum) was used. We then determined the minimum effective number of pulses to produce a detectable response in the artery by stimulating the nerve with trains of 1, 5, 6, 7, 8, 9, 10, 15, and 20 pulses (in random sequence) with a 2-minute rest between each of the trains; this required about 18 minutes.

Immediately thereafter, we applied 200 pulses to the nerve, waited 4 minutes, applied 200 pulses again, waited 4 minutes, and repeated the sequence of stimulation and rest for a total of 10 times; this required a little more than 40 minutes. A total of 2000 pulses were always given during this period. However, the individual trains of 200 pulses consisted in some experiments of stimuli at 2/second for 100 seconds and in others at 5/second for 40 seconds, 8/second for 25 seconds, 10/second for 20 seconds, 15/second for 13 1/3 seconds, 20/second for 10 seconds, or 25/second for 8 seconds.

The determination of the minimum effective number of pulses, immediately followed by the 10 sequences of 200 stimuli (2000 in all), required an hour. These procedures were then repeated every hour for a total of 8 hours or until the minimum number of effective number of pulses became unity.

In some of the preparations in which the minimum effective number of pulses became unity after repetitive nerve stimulation, the refractory period of this response was determined by the method of paired stimuli. Every 2 minutes two pulses were applied to the nerve at a progressively increasing interval until either a second contractile response was noted or the response to the first pulse was augmented. The shortest interval between two pulses that caused either of these two effects was taken as the refractory period. This measurement was confirmed by repeating the experiment commencing with an interval greater than the previously determined period and reducing this interval until the second pulse became ineffective.

A second series of experiments was identical to those described above except that transmural stimulation was used instead of nerve stimulation. The duration of the transmural pulses was 0.3 msec. Supramaximal voltage varied between 20 and 60 volts.
Results

Minimum effective number of pulses before repetitive nerve stimulation.—The relation between the number of pulses in a train and the magnitude of the contractile response in a typical experiment is illustrated in Figure 2 (crosses). No contractile response was recorded until a train of 7 pulses was given. An increase in the number of pulses beyond this caused a corresponding increase in response. In the series of 52 experiments reported in this study (Fig. 3), the minimum effective number of pulses varied between 5 and 9; the mode was 7. If the minimum effective number of pulses was determined hourly and the tissue allowed to rest between each determination, its numerical value remained constant in any one tissue throughout 8 hours.

Minimum effective number of pulses after repetitive nerve stimulation.—When the arterial segment was subjected to repetitive nerve stimulation (2000 stimuli every hour; see plan of experiment), the minimum effective number of pulses invariably changed to unity if the frequency of pulses was greater than 10/second. In the experiment illustrated in Figure 2 (solid circles) this change occurred after 2000 repetitive pulses at 25/second. When the minimum effective number of pulses became unity, the magnitude of the responses to trains of 2 to 7 pulses did not increase. However, with pulse trains longer than the original minimum effective number of pulses (before any repetitive nerve stimulation), the response increased approximately in parallel with the control. These changes were characteristic of all those encountered in this series. This effect persisted for the duration of the experimental period.

The number of hours necessary to effect this change in the minimum effective number of pulses was determined at different frequencies of repetitive stimulation (Fig. 3). The change was seen invariably after 1 hour (a total of 2000 pulses) when the pulses were delivered at 25/second. It did not occur within 6 hours (a total of 12,000 pulses) when these were delivered at 2, 5, or 8/second. It occurred only occasionally when the 2000 stimuli/hour were delivered at 10/second, and after an average of 2 hours when they were delivered at 15/second. Stimulation frequency was the only variable in these experiments. The changes observed with nerve (crosses) and transmural (solid circles) stimulation are presented on the same figure.

When the minimum effective number of pulses became unity, the refractory period was determined in eight preparations in which the rapid, spontaneous changes in tension (see below) were minimal. This facilitated the determination. The refractory period varied between 15 and 30 seconds. Since refractory periods of this length are several orders greater than those generally encountered before repetitive stimulation, no attempt was made to measure them more precisely.

Other changes.—Coincident with or just preceding the change in minimum effective number of pulses, the resting tone of the artery, as shown by the recorded baseline between stimulation periods, slowly but definitely increased. At the same time there were small, fairly rapid, spontaneous changes in tension (Fig. 4).

All changes consequent upon repetitive nerve stimulation persisted after replacement of the modified Krebs solution in the tissue.
bath and occurred when the solution was replaced hourly during the course of an experiment.

Yohimbine (10 µg/mg) abolished all responses to nerve stimulation and also the small rapid fluctuations in tone. In only 6 out of 10 experiments did it reverse or partly reduce the increase in resting tone.

**Effect of various pharmacological agents on the minimum effective number of pulses.** Since adrenergic nerve terminals are known to take up catecholamine from the extracellular space, arterial preparations were exposed to a subthreshold (0.002 µg/ml) and a suprathreshold (0.02 µg/ml) concentration of l-norepinephrine in the tissue bath; a threshold concentration causes a just-detectable contraction of the artery. The minimum effective number of pulses was determined before and during exposure to l-norepinephrine and 1 hour after its removal by washing the preparation. On no occasion did such exposure from 30 minutes to 2 hours alter the minimum effective number of pulses (P = 0.95). Furthermore, pretreatment with cocaine, which presumably inhibits the reuptake of the transmitter (6), was without influence on the minimum effective number of pulses; the concentration used (2 µg/ml) was one that causes optimal potentiation of the contractile response to nervous stimulation and iproniazid (2 µg/ml) and pyrogallol (4 µg/ml) in concentrations which in other tissues inhibit monoamine oxidase (7) and catechol-O-methyl transferase respectively (8). In each instance, five experiments were carried out; P was 1.0, 0.85, and 0.90, respectively.

**Discussion**

Our experiments show that in vitro the sympathetic nerve to the pulmonary artery must be stimulated with a train of 5 to 9 pulses before contraction may be recorded and that repetitive high, but not low, frequency stimulation reduces this value to unity. If the significance of this change is to be understood, the underlying cause of a minimum effective number of pulses of 5 to 9 must be appreciated. Our previous observations suggest that this is not the consequence of an insensitive tension-recording system, nor of the physical characteristics of the vessel wall (2).

The transmitter-containing nodes of the terminal autonomic plexus in this blood vessel are situated at a minimum distance of 4,000 Å from the closest layer of vascular muscle cells (9). Thus, the released transmitter may reach the effector cells in very low concentration. The minimum effective number of pulses may therefore be related to the concentration necessary to activate the alpha-receptors. Such an explanation is consistent with the electrophysiological findings of Burnstock and Holman (10), who found that in the vas deferens the summated depolarization of 5 to 8 pulses was needed before an action potential was initiated in the effector cells. However, if the same phenomena were responsible for the minimum effective number of pulses in this artery, then this should be reduced by exposure of the tissue to a low concentration of the transmitter. We have assumed in this case that the transmitter is l-norepinephrine (11). Our experiments demonstrated, however, that the minimum effective number of pulses was uninfluenced by exposure to l-norepinephrine under a variety of circumstances.

A minimum effective number of pulses of 5 to 9 may be the consequence of the partial depletion of transmitter caused by hypoxia and handling of the tissue, the rapid destruction of the transmitter by metabolizing enzymes, or its rapid reuptake at the terminal autonomic plexus. Our studies with l-norepinephrine under conditions that should have replenished the depleted transmitter, with pyrogallol and iproniazid, which inactivate the transmitter metabolizing enzymes, and with cocaine, which inhibits the reuptake of the transmitter at the nerve terminal, suggest that none of these explanations is sufficient.

Norepinephrine is released by nervous activity from storage sites, probably subcellular vesicles in the nodal swellings of the terminal effector plexus. It is speculated that the 5 to

**Abnormal Transmitter Release**
9 pulses may be needed to cause the mobilization and migration of the subcellular vesicles to the neurilemma (12), and subsequent release of sufficient transmitter to effect a detectable response. We propose, therefore, that the minimum effective number of pulses reflects a presynaptic phenomenon.

Our experiments have shown that repetitive stimulation with up to 12,000 pulses (2000/hour for 6 hours) at frequencies of 10/second or less are without effect on the normal pattern of contractile response of the tissue. Stimulation with frequencies higher than 10/second, which is the upper limit of rates encountered under physiological conditions, caused changes which did not reverse during the course of our experiments. Furthermore, the number of pulses needed to effect this change was frequency-dependent.

The abnormal response of the vessel to a single stimulus is probably the result of a facilitated abnormal release of the transmitter. The extraordinarily long refractory period of this response (15 to 30 seconds) attests to its "abnormal" nature. If a preparation in which this change has occurred is stimulated for 2 seconds at 10/second, then pulses 2 through 20 must fall within the refractory period of the response to the first pulse. Consequently, they cannot act by the same mechanism as the first pulse. However, since a train of 20 pulses does cause a contractile response greater than that following a single pulse (Fig. 2), two independent mechanisms must be involved. The first is an "abnormal" response to the first of a train of pulses. The second is probably a "normal" mechanism which releases the transmitter when trains greater than the minimum effective number of pulses are used. Thus, the curve shown in Figure 2 (solid circles) represents the contractile response produced by transmitter released by two separate mechanisms superimposed upon each other.

Two other phenomena were associated with this change in minimum effective number of pulses. The rapid spontaneous fluctuations in tone appear to be the consequence of adrenergic transmitter release since they were invariably antagonized by an alpha-adrenergic receptor-blocking agent. If the response to a single stimulus represents a facilitation of transmitter release, an extension of the same process would result in the spontaneous release of the transmitter. The rapid fluctuations in tone may be due to such a release. As the slow sustained increase was inconstantly affected by yohimbine, the nature of this effect must await further analysis.

The significance of these observations is unknown. All three changes that occur after repetitive high frequency stimulation cause an increase in effector response to nervous activity. Kubicek et al. (13) stimulated the splanchnic nerves of a rabbit at low frequencies, 20 hours a day for 38 days, without causing a permanent increase in arterial pressure. No studies at high frequencies were made. Bazanova et al. (14) found that short-duration (2 minutes) stimulation of preganglionic fibers in the frog led to degenerative morphological changes in the nerve cells only when frequencies of 10 or more were used. They state that "the cytoplasm in the body of the neuron became coarsely granular. The nerve cell generally became darker and the pericellular apparatus stained more intensely with methylene blue and silver salts." Such changes persisted for several hours but were reversible. The consequences of more prolonged stimulation were not described.

Although the conditions of these studies were quite different from our experiments, they do show that morphological neuronal changes can occur in vivo when stimulation frequencies greater than 10/second are used.

References


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