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Noradrenaline-Induced Restoration of Acidosis-Inhibited Neurogenic Vasoreactivity at Using Different Electrical Stimulation Frequencies

V. N. Yartsev and O. V. Karachentseva

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Experiments using segments of the tail artery from young rats addressed the effects of noradrenaline on the neurogenic vasoconstrictor reactions of these segments evoked by stimulation with an electric field of frequencies 3, 5, 10, and 40 Hz in controls and after decreasing the solution pH from 7.4 to 6.6. Acidosis produced significant decreases in this reaction at all electrical stimulation frequencies. Noradrenaline restored neurogenic vasoconstriction after it had decreased spontaneously or after the significantly greater drop induced by acidosis. On the background of acidosis, the potentiating action of noradrenaline on neurogenic vasoconstriction was more significant than at normal pH and was more apparent at higher electrical stimulation frequencies and noradrenaline concentrations. This may be of value for redistributing blood flow to vitally important areas during muscle work, which is accompanied by acidosis, increased spike frequencies in sympathetic nerves, and increases in noradrenaline concentrations.

Keywords: rat tail artery, noradrenaline, electrical field stimulation, acidosis.

The mechanisms regulating blood flow can be divided into primary, hormonal, and local [36], which in in vivo conditions interact closely with each other, the result of this interaction very often being unpredictable. Studies of this interaction are of great importance for understanding the operating principles of the cardiovascular system. Central neurogenic regulation of the circulation is known to involve neurogenic vessel tone; this, however, can undergo significant changes in response to various factors, resulting in changes in the effectiveness of the neurogenic control of the circulation. In particular, these factors include the hormonal background and the pH of both the blood and the tissue fluid. Identification of the action of noradrenaline – a hormone present in the plasma at concentrations which can vary significantly in response to various changes in the body's physiological status – on neurogenic tone is of interest for understanding the principles of the interaction between nervous and hormonal mechanisms of regulation of the circulation. Thus, for example, the blood noradrenaline concentration during stress can increase, according to various reports, by factors of 6–11 [7, 32, 34], reaching values of 0.04 [32] and even 0.27 μM [7]. A small number of publications have described the action of noradrenaline on neurogenic vasoconstriction and have shown that noradrenaline suppresses this reaction [6] or induces vasodilation [30] or a biphasic reaction [31] in response to stimulation of the perivascular nerves. Only one study found a potentiating effect for noradrenaline on neurogenic contraction of blood vessels [37]. Our studies of the action of noradrenaline on neurogenic vasoconstriction provided the first evidence that noradrenaline can restore neurogenic vessel contraction after its spontaneous or acidosis-induced decline [4]. Blood/tissue acidosis, or decreased pH, is known to be one of the most important vasodilating factors contributing to the local mechanisms regulating the circulation. Our previous study [1] showed that after a small spontaneous decrease in the neurogenic vasoconstrictor reaction, the greatest level of its potentiation occurred in response to low noradrenaline con-

Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia; e-mail: yartsev@infran.ru.

centrations, while after significant drops in the neurogenic response due to acidification of the medium, the largest and most significant potentiation was seen with higher noradrenaline concentrations. These data were obtained using vessel electrical stimulation frequencies of 10 Hz. It is known from published data that activity in sympathetic nerves consists of individual spikes or volleys of 2–7 spikes with interspike intervals corresponding to a frequency of 20–50 Hz [23], while in stress this increases significantly, particularly in the rat tail artery in cold stress, from 0 to 250 spikes over 15 sec, which corresponds to a mean frequency of 17 Hz [33]. The electrical stimulation frequency determines which mechanisms will support vessel contraction [19, 35, 39, 40], so there is interest in evaluating the potentiating action of noradrenaline on neurogenic vasoconstriction in conditions of acidosis at different electrical stimulation frequencies.

Methods

Experiments were performed on male Wistar rats (n = 46) aged 4–6 weeks and weighing 70–120 g. Animals were anesthetized with ether and the tail artery was prepared; a ring of length 1.2 mm was excised from the proximal part. The arterial segment was placed on two tungsten needles (each of diameter 70 μm), one of which was connected to the rod of a microelectrode inserter and the second to a 6MKh1S mechanotron, the signal from which was amplified and delivered to a KSP-4 recorder which documented changes in tension in the segment wall. The needles with the ring segment were then placed in a thermostatted (36°C) flow bath of volume 10 ml filled with bicarbonate Krebs solution containing 115 mM NaCl, 4.7 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4.7H_2O$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 10 mM glucose. Solution pH was adjusted to 7.4 and monitored throughout the whole experiment. After adaptation of the preparation for 30 min at a level of stretching corresponding to a force of about 0.8 mN, the myograph needles were approximated and then separated in steps until the tension giving the maximal response of the vessel segment to electrical field stimulation (30 V, 3 msec, for 3 sec) at a frequency of 5 Hz was reached, after which it was stimulated, with 3-min intervals, at frequencies of 10, 3, and 40 Hz, which correspond to the frequencies in sympathetic nerves in vivo [33]. Electrical stimulation was performed using two platinum electrodes positioned 2 mm from the vessel segment. Our previous experiments using tetrodotoxin (0.1 μM) showed that in our methodological conditions, these electrical stimulation parameters produced vessel contraction mainly due to stimulation via nerve fibers [18]. During the following 120 min, the vessel was stimulated every 3 min at a frequency of 5 Hz and after 120 min electrical stimulation was performed at frequencies of 10, 3, and 40 Hz with 3-min intervals; electrical stimulation was then terminated and noradrenaline bitartrate (Sigma, Steinheim, Germany) was added to concentrations increasing cumulatively from 0.03 to 10 μM (in the bath). After reaching the maximum tonic contraction of the vessel wall (for each noradrenaline concentration), a series of electrical stimuli was delivered at frequencies of 5, 10, 3, and 40 Hz. The second series of experiments differed from the one described above only in that solution pH was decreased from 7.4 to 6.6 using CO_2 90 min after stretching the segment and was kept at this level to the end of the experiment. The pH was decreased using CO_2 as a physiologically appropriate and widely used method (see, for example, Dabertrand et al. [15]). Solution pH was kept constant by intermittent passage of the required quantity of CO_2 and room air containing 21% O_2 through the solution. The normal functioning of the rat tail artery is known to be supported by the amount of oxygen present in a mix of 16% O_2 + 5% CO_2 + 79% N_2 [27]. The magnitude of the initial response to electrical stimulation at 10 Hz 5–10 min after stretching the segment was taken as 100%.

Results were analyzed statistically by identifying significant differences by paired (within-group) or unpaired (between-group) comparisons using Student's t test ($p < 0.05$).

Results

In the first series of experiments, performed at 36°C and pH 7.4, most cases, as in our previous studies [1, 2, 4] in young rats, showed spontaneous decreases in electrical stimulation-induced contractile responses, which were restored to the initial value by noradrenaline at concentration of 0.03–0.5 μM (Fig. 1, *A* and Fig. 2). In particular, the magnitude of the contractile response to electrical stimulation at 5 Hz, amounting to 3.8 ± 0.3 mN at the beginning of the experiment, dropped to 2.6 ± 0.3 mN at 120 min ($p < 0.0005$) and increased in the presence of 0.03 μM noradrenaline to 3.5 ± 0.3 mN, which was not significantly different from the baseline value ($p > 0.05$). When the pH was reduced from 7.4 ± 6.6, there was no consistent change in vessel tone, though the neurogenic response decreased significantly (Fig. 1, *B*), reaching (after changing the pH) a magnitude of, for example, 42.5 ± 3.8% of baseline at a frequency of 10 Hz at 30 min (Fig. 2), which was almost two times smaller than the spontaneous decrease in the response in controls at pH 7.4 (77.6 ± 4.1%). This response increased in the presence of noradrenaline. Figure 2 shows that neurogenic responses at pH 6.6 were significantly smaller than at pH 7.4 for all electrical stimulation frequencies both before and on the background of noradrenaline at concentrations of 0.03–0.1 μM. However, the potentiating action of 0.5 μM noradrenaline on the neurogenic response in acidosis was so strong that it reached the value obtained at pH 7.4. To compare the potentiating actions of noradrenaline at different pH values, plots were constructed for each electrical stimulation frequency (Fig. 3), which show that the potentiating actions of noradrenaline at concentrations of 0.03–0.05 μM at an electrical stimulation frequency of 3 Hz on the background of acidosis were smaller, while at other concentrations the action was similar to that in normal pH conditions. At an electrical stimulation frequency of 5 Hz, the potentiating action of 0.03 μM noradrenaline on the background of acidosis was smaller, while actions at concentrations of 0.05 to

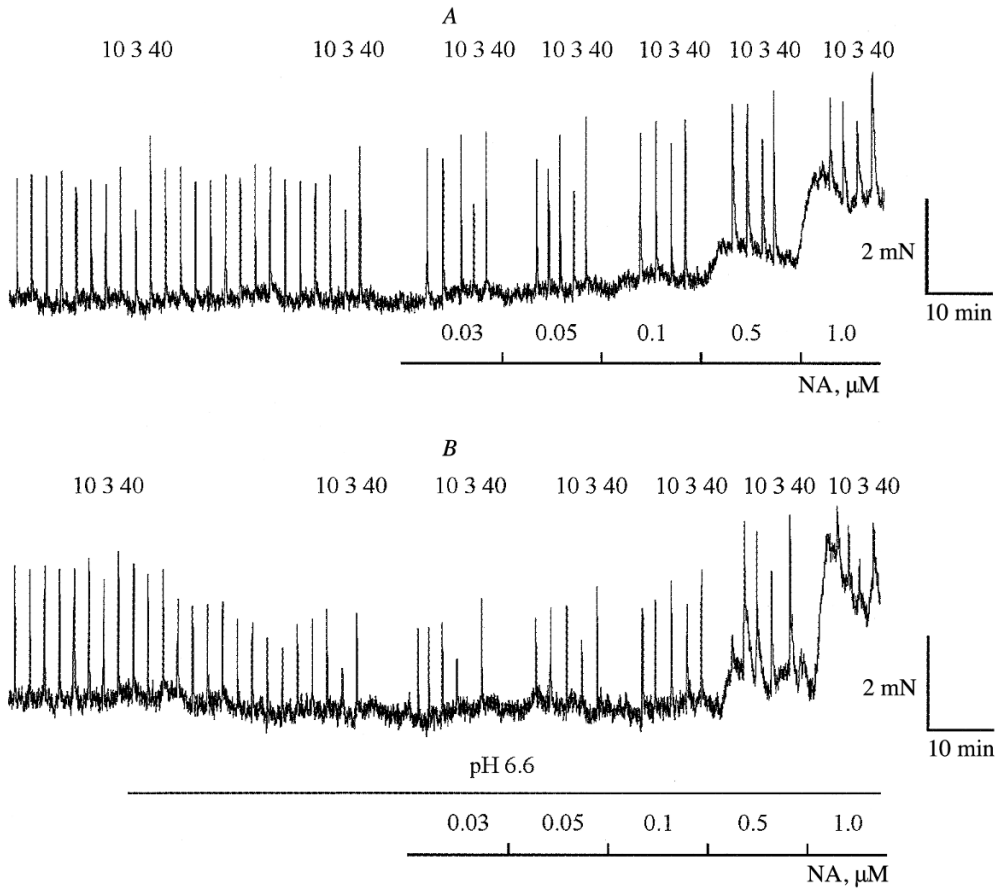

Fig. 1. Mechanograms of rat tail artery segments demonstrating contractile reactions to stimulation with an electric field before and during exposure to noradrenaline (NA) at solution pH 7.4 (*A*) and 6.6 (*B*). Numbers above mechanograms show electrical stimulus frequency (Hz) for the corresponding contractile responses. The electrical stimulation frequency for the remaining contractile responses peaks was 5 Hz.

0.1 μM were the same as, and actions at concentrations of 0.5–1.0 μM were greater than those at normal pH. At higher electrical stimulation frequencies, the potentiating action of 0.03–0.1 μM noradrenaline on the background of acidosis was the same, while that at a concentration of 1.0 μM was greater than that on the background of normal pH; at an electrical stimulation frequency of 40 Hz, there was a significant difference only for this noradrenaline concentration. Thus, on the background of acidosis, the potentiating action of noradrenaline on the neurogenic response at each electrical stimulation frequency improved with increases in the noradrenaline concentration as compared with the potentiating action seen at normal pH, becoming significantly more marked at high frequency and high concentrations. It should be noted that in acidosis, the maximum potentiation for all electrical stimulation frequencies was seen at a noradrenaline concentration of 0.5 μM, while at pH 7.4 this occurred at 0.1 μM and potentiation at frequencies of 5–10 Hz was significantly greater ($p < 0.05$). It is interesting that the potentiating effects of 0.1 μM noradrenaline were virtually identical regardless of solution pH and electrical stimulation frequency. The question of identifying the electrical stimulation frequencies at which the potentiating action of noradrenaline on the neurogenic response is most significant was addressed by constructing plots (Fig. 4) which show that in acidosis, the greatest suppression of neurogenic vasoconstriction occurred at electrical stimulation frequencies of 5 and 10 Hz, and the greatest level of potentiation by 0.03–0.5 μM noradrenaline was obtained at electrical stimulation frequencies of 5–40 Hz. At the same time, at normal pH there was no significant relationship between the effectiveness of the potentiating action of noradrenaline and electrical stimulation frequency at any noradrenaline concentration, with the exception of 0.03 μM, at which the neurogenic response was significantly greater than at 3 Hz.

Discussion

The studies reported here show that the potentiating action of noradrenaline on neurogenic vasoconstriction in conditions of acidosis has the following features as compared with the action at pH 7.4: 1) at electrical stimulation

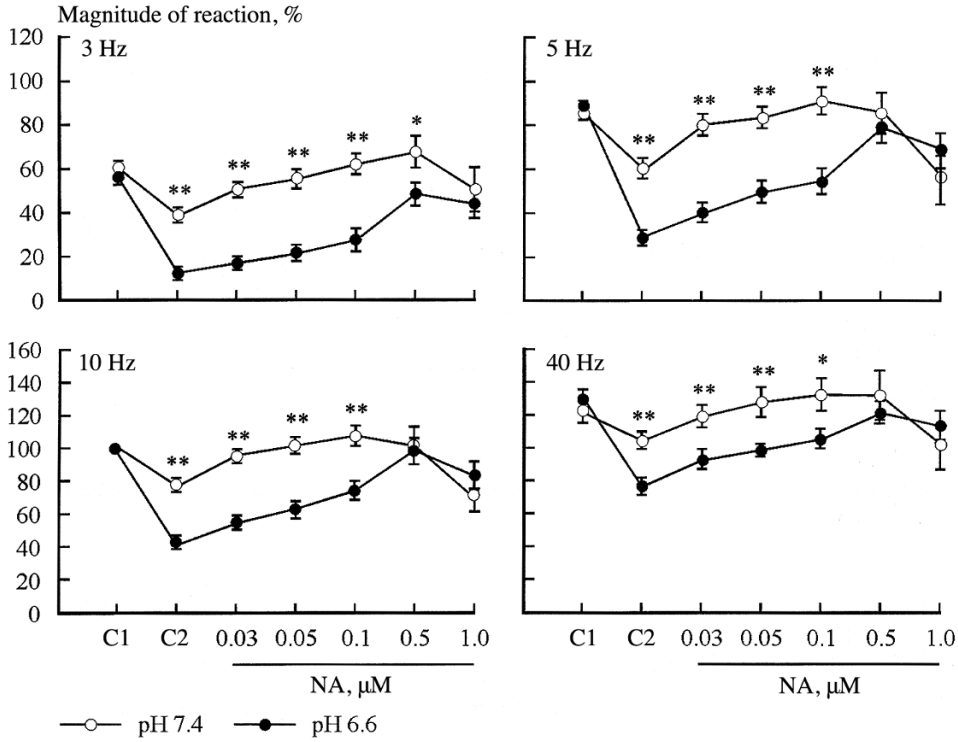

Fig. 2. Contractile reactions of rat tail artery segments to electrical stimulation at frequencies of 3–40 Hz 5–10 min (C1) and 120 min (C2) after stretching of the segment in controls and on the background of exposure to noradrenaline (NA) at concentrations of 0.03–1.0 μM at pH 7.4 (n = 22) and 6.6 (n = 24). The ordinate shows the magnitudes of reactions compared with the baseline value at an electrical stimulation frequency of 10 Hz. Here and henceforth, data are shown as mean ± standard error of the mean. Significant differences: $*p < 0.05$, $**p < 0.01$ compared with pH 6.6.

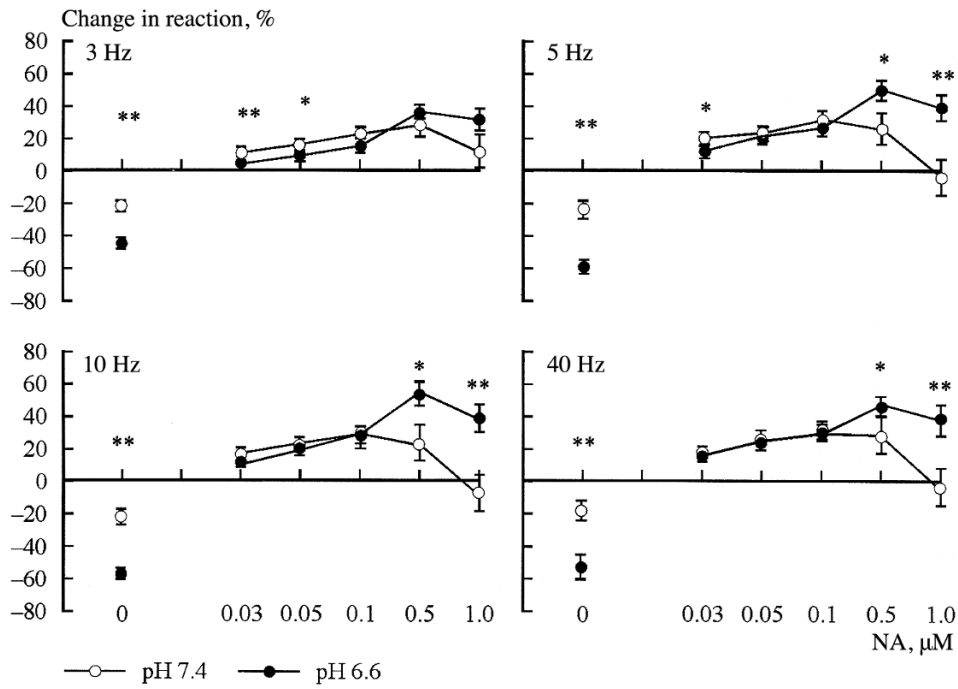

Fig. 3. Changes in the contractile reactions of rat tail artery segments to electrical stimulation at frequencies of 3–40 Hz in the presence of noradrenaline (NA) at concentrations of 0.03–1.0 μM at pH 7.4 (n = 22) and 6.6 (n = 24). The ordinate shows changes in reactions (0 NA – 120 min after stretching the segment compared with baseline; in the presence of NA – compared with reaction magnitude immediately before addition of NA). Initial contractile reaction magnitude at an electrical stimulation frequency of 10 Hz was taken as 100%. Significant differences: $*p < 0.05$; $**p < 0.01$ compared with pH 6.6.

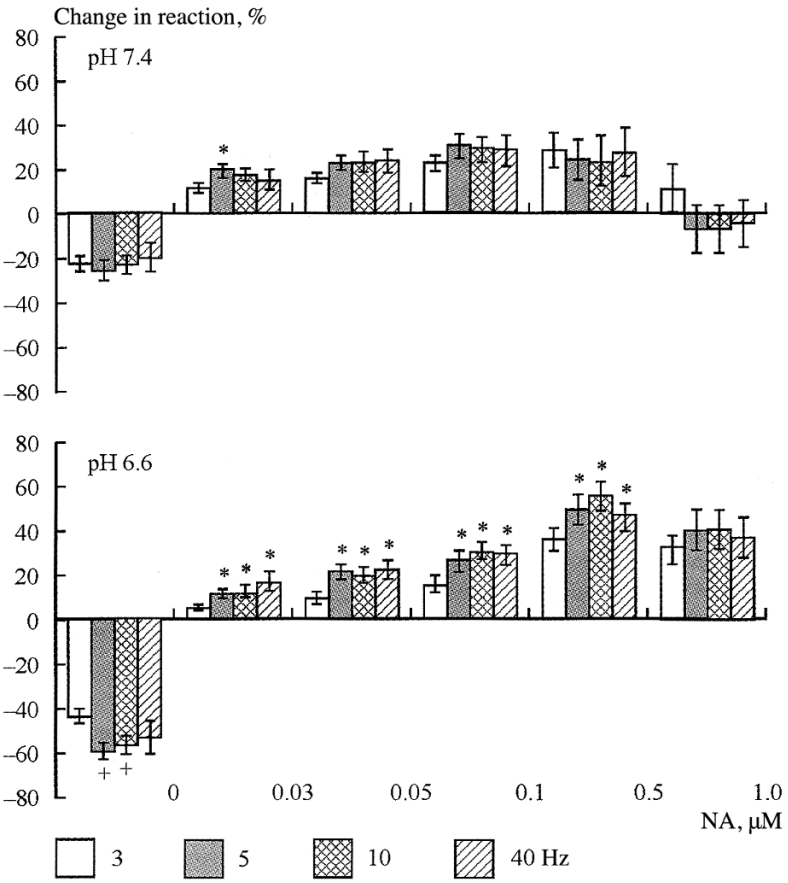

Fig. 4. Relationship between the potentiating action of noradrenaline (NA) on contractile reactions of rat tail artery segments evoked by electrical stimulation and the frequency of this stimulation at pH 7.4 ($n = 22$) and 6.6 ($n = 24$). The ordinate shows changes in reactions (0 NA – 120 min after stretching the segment compared with baseline; in the presence of NA – compared with reaction magnitude immediately before addition of NA). Initial contractile reaction magnitude at an electrical stimulation frequency of 10 Hz was taken as 100%. Significant differences: $^{*}p < 0.05$, $^{+}p < 0.005$ compared with 3 Hz.

frequencies of 5–40 Hz, potentiation was maximal at higher noradrenaline concentrations; 2) for all electrical stimulation frequencies, the potentiating action improved with increases in the noradrenaline concentration; 3) at electrical stimulation frequencies of 5–40 Hz, potentiation was greater than at 3 Hz. That is, in conditions of acidosis, the potentiating action of noradrenaline on neurogenic vasoconstriction was more apparent at higher electrical stimulation frequencies and noradrenaline concentrations.

It is known from published data that the contractile responses of isolated rat tail arteries to exogenous noradrenaline is due mainly to stimulation of α_{1A}-adrenoreceptors on smooth muscle cells and, to a lesser extent, α_{1B}- and α_{1D}-adrenoreceptors [24]. However, smooth muscle cells in the rat tail artery also bear α_2-adrenoreceptors, which are involved in cold vasoconstriction [22], while their endotheliocytes bear α_1-adrenoreceptors [16], which trigger the process of vasodilation [29]. In addition, presynaptic α_2- and β-adrenoreceptors are known to be present, stimulation of the former leading to decreases and stimulation of the latter leading to increases in noradrenaline and ATP release from the perivascular nerves [9, 10].

Few previous studies of the action of noradrenaline on neurogenic contraction of blood vessels have been reported by other authors. In particular, studies of human artery have shown that electrical stimulation at frequencies of 2–8 Hz on the background of 0.1–0.3 μM noradrenaline evokes dilation of the gastroepiploic artery [30], while on the background of 3 μM noradrenaline it induces a biphasic response of the uterine artery [31]. Decreases in constrictor and appearance of dilator responses are linked with nitric oxide release from NO-ergic nerve fibers. It should be noted that experiments on the gastroepiploic artery were preformed using guanethidine, which deprives nerve fibers of noradrenaline, while the noradrenaline concentration in experiments on the uterine artery was 3 μM, at which in our experiments at pH 7.4 the response to electrical stimulation was also suppressed, perhaps because most postsynaptic α-ad-

renoreceptors were already occupied in these conditions. Studies on rat tail arteries [6] showed that 0.01–0.1 μM noradrenaline decreased neurogenic reactivity, though electrical stimulation in this study was applied not only the background of noradrenaline (as in our experiments), but 3 min after the end of its action, and the authors linked this with a decrease in the sensitivity of postsynaptic receptors. It should be noted that in a recent study [5], increasing and repeated noradrenaline concentrations were not found to have any desensitizing action on α-adrenoreceptors in rat tail arteries, these being responsible for virtually the whole of the response of this artery to noradrenaline and nerve fiber stimulation. We found a single literature source in which the potentiating action of noradrenaline on neurogenic contraction of blood vessels was demonstrated. This was a report by Su [37], whose experiments on rabbit vessels showed that 0.1–1 μM noradrenaline significantly increased the responses of the mesenteric artery to electrical stimulation at a frequency of 8 Hz and produced a minor increase in the brachial artery, but decreased neurogenic constriction of the ear and pulmonary arteries. It should be noted that the potentiating action of noradrenaline in Su's experiments were accompanied by a decrease in its release from nerve endings and was independent of noradrenaline reuptake and of presynaptic β-adrenoreceptors. The author concluded that a possible mechanism for the potentiating effect of noradrenaline was partial membrane depolarization in vascular smooth muscle cells, which facilitates neurogenic contraction of these cells. It is possible that this mechanism may underlie the phenomenon of the restoring action of noradrenaline seen in our experiments, while the hyperpolarization occurring in acidosis [20] and arising as a result of factors including activation of the ASIC channels of vascular smooth muscle cells [14, 28] is the cause of the fact that at pH 6.6 only high noradrenaline concentrations produced sufficient depolarization to support neurogenic contraction.

The decrease in solution pH from 7.4 to 6.6 in our experiments evoked a significant decrease in neurogenic vasoconstriction at all electrical stimulation frequencies. Despite the fact that acidosis has long been regarded as a classical vasodilating agent in the metabolic component of blood flow regulation, the effects of acidosis on neurogenic vascular tone have received virtually no study. There are only data on minor suppressive effects of acidosis on neurogenic contractile reactions of vessels in perfused small intestine [13] and the femur in dogs [17]. In addition, studies on isolated dog subcutaneous veins in acidosis showed a decrease in the magnitude of the response of these vessels to electrical stimulation of their nerves [38]. Our previous studies of this question using isolated rat mesenteric [3] and tail [2] arteries provided the first evidence of the suppressive effects of acidosis on the neurogenic contractile reactions of these vessels evoked by electrical stimulation at 10 Hz. This effect was to a significant extent due to an increase in NO synthesis [1], though it may also be mediated by various mechanisms associated with the operation of nerves, endothelial, and smooth muscle cells [12]. In particular, there may be an increase in the quantity of vasodilatory mediator in acidosis because of activation of TRPV1 vanilloid receptors of CGRP-ergic nerves [25], with decreases in the actions of released vasoconstrictor mediator on smooth muscle cells via postsynaptic α-adrenoreceptors [21], and increases in NO release from both endothelial and smooth muscle cells [11].

In our experiments, the potentiating effect of noradrenaline on the background of acidosis was more marked at electrical stimulation frequencies of 5–10 Hz than at 3 Hz. The cause of this may be that the ratio of mediators released from sympathetic nerves at low electrical stimulation frequencies increases in favor of ATP [13, 35, 39, 40], while the sensitivity of postsynaptic P2X purine receptors, in contrast to postsynaptic α_1-adrenoreceptors, decreases significantly on the background of acidosis [26].

We have shown that in acidosis, the potentiating action of noradrenaline on neurogenic vasoconstriction is more apparent at higher electrical stimulation frequencies and noradrenaline concentrations. The state of acidosis is known to arise particularly as a result of physical work, which is accompanied by increases in spike frequencies in sympathetic nerves and increases in noradrenaline concentrations [7]. It can be suggested that the phenomenon seen here is important for the redistribution of blood flow from less vitally important areas, such as those fed by the rat tail artery, to areas with greater importance for survival in conditions of the active use of skeletal muscles.

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