The Relaxing Effects of Barbiturates in Vascular Smooth Muscle of Rat Aorta

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The effects of thiamylal and pentobarbital on contractions mediated through the influx of extracellular Ca^{++} and the release of intracellularly stored Ca^{++} were compared in rat aortic strips. Thiamylal (3×10^{-5}M to 10^{-3}M) and pentobarbital (10^{-4} to 10^{-3}M) significantly attenuated the contraction induced by KCl (20 mM), and shifted the dose-response curve for Ca^{++} of KCl (20 mM)-treated strips downwards and to the right. Caffeine (10^{-2}M)-induced contraction was significantly attenuated by thiamylal at concentrations greater than 10^{-4}M and by pentobarbital at 3×10^{-4}M. Only a high concentration (10^{-3}M) of these barbiturates significantly inhibited the contractions induced by norepinephrine (NE, 10^{-6}M) in Ca^{++}-free medium. Contraction of strips without endothelium by a Ca^{++} ionophore, A23187 (5×10^{-6}M), in the presence of a Ca channel blocker, was relaxed by high concentrations of thiamylal (3×10^{-4}M to 10^{-3}M) and pentobarbital (10^{-3}M). It is concluded that thiamylal inhibits contraction through an intracellular action as well as a Ca channel-blocking action in vascular smooth muscle of rat aorta. However, the intracellular action of pentobarbital is less potent than that of thiamylal. (Key words: anesthetics, intravenous-pentobarbital, thiamylal, muscle, smooth-vascular, arteries-aorta)

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Barbiturates have been shown to inhibit contractions induced by the influx of Ca^{++} through voltage-dependent and receptor-operated channels in vascular smooth muscle of various origins[1–10]. On the other hand, a recent study we have performed using dog mesenteric artery[11] has demonstrated that thiamylal strongly inhibits contractions mediated through the release of intracellularly stored Ca^{++} elicited by norepinephrine (NE) and caffeine, as well as contractions of chemically skinned fibers elicited by the addition of Ca^{++} to the bathing fluid, suggesting that the relaxing effect of thiamylal in these arteries occurs largely through intracellular action. However, it is known that the actions of vasoactive agents on vascular smooth muscle often differ according to species or the site of the vessels[12,13]. Furthermore, the vascular actions of oxybarbiturates may differ from those of thiobarbiturates[7,8,13,14]. In the present study, therefore, we examined the effects of thiamylal and pentobarbital on contractions mediated through Ca^{++} release and those by application of Ca^{++} ionophore, in addition to those mediated through Ca^{++} influx, in an attempt to reveal the main site of action of these barbiturates in the vascular smooth muscle of rat aorta.

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Methods

The protocol was approved by the Kyoto University Animal Use Committee. Male

Wistar rats weighing 250 to 350g were anesthetized by intraperitoneal injection of sodium pentobarbital, 50 $mg \cdot kg^{-1}$, and killed by exsanguination. The chest of each rat was opened, and the descending portion of the thoracic aorta was isolated and cut into helical strips approximately 17 mm long. Each strip was then bathed in a 10-mL organ bath containing Krebs' bicarbonate solution, with the composition (in mM); NaCl 118.2, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.8 and dextrose 10. The bathing fluid was aerated with a mixture of 95% O_2 and 5% CO_2 to keep the pH of the solution within the range of 7.35 to 7.45, and maintained at 37 ± 0.5°C. Each strip was vertically fixed between two hooks, and the hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Toyo Baldwin T7-240, Japan); changes in isometric tension were displayed on an ink-writing oscillograph (Rectigraph 8K, Nihondenki Sanei Co., Japan).

The resting tension was adjusted to 1.0g and each arterial strip was allowed to equilibrate for 90–120 min in the control medium, during which time the fluid was changed every 15 min. Then contraction was induced by 30 mM KCl, followed by washing three times with fresh medium.

KCl- and norepinephrine-induced contractions

Contractions were induced repeatedly with KCl (20 mM) or norepinephrine (NE, 10^{-6}M) in normal medium until the tension induced by the constrictor did not change during two successive applications. Between measurements, the preparation was washed more than three times with fresh medium for 30–40 min. In all strips, sustained contractions were induced by these concentrations of constrictors, although in some strips a rapid-developing, transient contraction preceded the sustained contraction. To examine the effects of thiamylal and pentobarbital on the contractions induced by KCl or NE in normal medium, each arterial strip was exposed to different concentrations of thiamylal or pentobarbital, or an equal volume of distilled water (control study) for 20 min, and the change in tension induced by the constrictor in steady state was measured. To examine the effect of thiamylal and pentobarbital on the contraction induced by NE in Ca^{++}-free medium, each strip was exposed to different concentrations of thiamylal and pentobarbital for 10 min in normal medium, and then bathed in Ca^{++}-free medium with 2 mM EGTA containing the same concentration of thiamylal or pentobarbital for 10 min before exposure to NE.

Dose-response curve for Ca^{++} of KCl-depolarized strips soaked in Ca^{++}-free medium

To examine the effects of thiamylal and pentobarbital on contractions induced by Ca^{++} influx in KCl-depolarized strips, the response to 20 mM KCl was first measured in normal medium, and then the strip was exposed to Ca^{++}-free medium containing 0.1 mM EGTA for 40 min, during which time the fluid in the bath was replaced every 20 min.

Subsequently, the strip was exposed to 10^{-4}, 3×10^{-4} or 10^{-3}M thiamylal or pentobarbital, or to an equal volume of distilled water (control study), and after another 10 min, 20 mM KCl was added to the bathing medium. After a further 10 min (i.e. after 60 min of exposure to Ca^{++}-free medium), Ca^{++} (0.5–10 mM) was added to the medium in a cumulative manner.

Caffeine-induced contraction

Arterial strips were treated with 10^{-2}M caffeine repeatedly in Ca^{++}-free medium containing 2 mM EGTA to deplete their intracellularly stored Ca^{++}, then loaded with Ca^{++} by bathing them for 20 min in Krebs bicarbonate solution containing 2.5 mM Ca^{++}, and subsequently exposed to Ca^{++}-free medium containing 2 mM EGTA for 10 min. After these treatments, the strips were exposed to caffeine (10^{-2}M). For treatment with barbiturates, thiamylal or pentobarbital (3×10^{-5} to 10^{-3}M) was added to the Ca^{++}-free medium for 10 min before

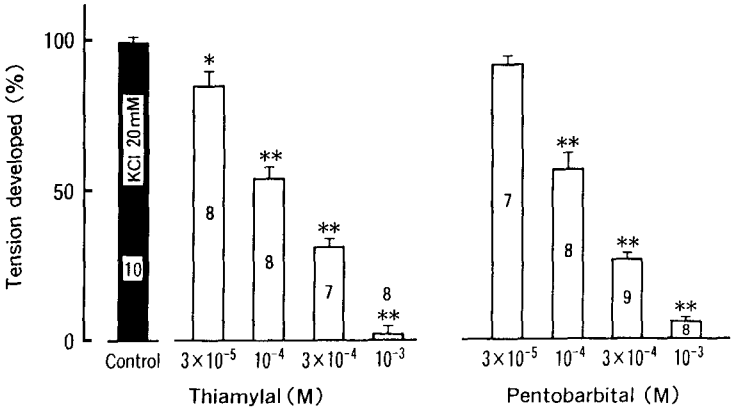

Fig. 1. Modification by thiamylal and pentobarbital of contraction induced by KCl (20 mM) in rat aortic strips. Contraction was induced by 20 mM KCl after 20 min of treatment with thiamylal, pentobarbital (3×10^{-5} to 10^{-3}M) or an equivalent volume of distilled water (control). KCl (20 mM)-induced contraction before treatment was taken as 100%, the absolute values of which averaged 411 ± 31 mg (n = 17). Values in the bars indicate the numbers of strips studied. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control.

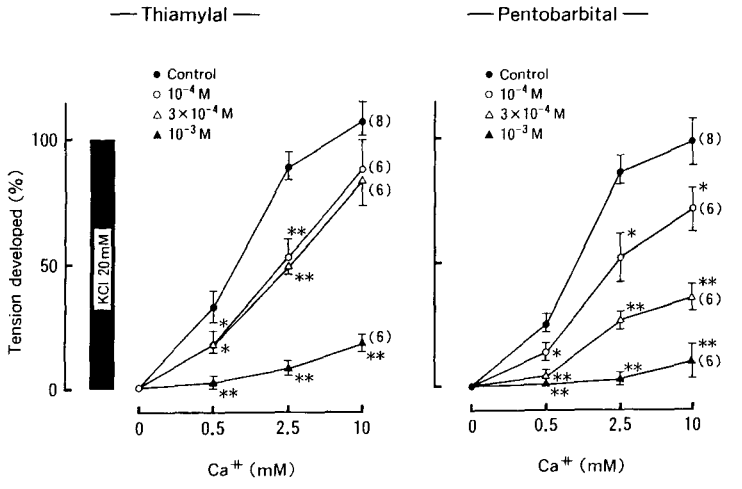

Fig. 2. Modification by thiamylal and pentobarbital (10^{-4} to 10^{-3}M) of the Ca^{++} dose-response curve of KCl-depolarized strips which had been soaked in Ca^{++}-free medium for 60 min. KCl (20 mM)-induced contraction in normal Ca^{++} medium was taken as 100%, the absolute values of which averaged 653 ± 37 mg (n = 20). Values in parentheses indicate the numbers of strips studied. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control.

exposure to caffeine.

Effects of thiamylal and pentobarbital on strips precontracted with KCl and Ca ionophore

The endothelium of the strips used for this study was removed mechanically to avoid any involvement of endothelium-mediated mechanisms, since Ca ionophore is known to activate the formation of endothelium-dependent relaxing factor (EDRF)[15]. The inner surface of each strip was rubbed with a cotton ball, and removal of the endothelium was confirmed pharmacologically by the absence of any relaxing response to acetylcholine (10^{-6}M) in strips precontracted with KCl (20 mM)[15]. Each strip was then washed more than three times with fresh medium and contracted with 20 mM KCl or, after pretreatment with nifedipine (5×10^{-7}M), contracted with the Ca ionophore, A23187 (5×10^{-6}M). When the tension had stabilized, thiamylal or pentobarbital at a concentration of 10^{-5} to 10^{-3}M was added to the medium in a cumulative manner, and finally papaverine (10^{-4}M) was added to obtain the maximum relaxation. The degree of relaxation induced by barbiturates was expressed as a percentage of that induced by papaverine.

Drugs

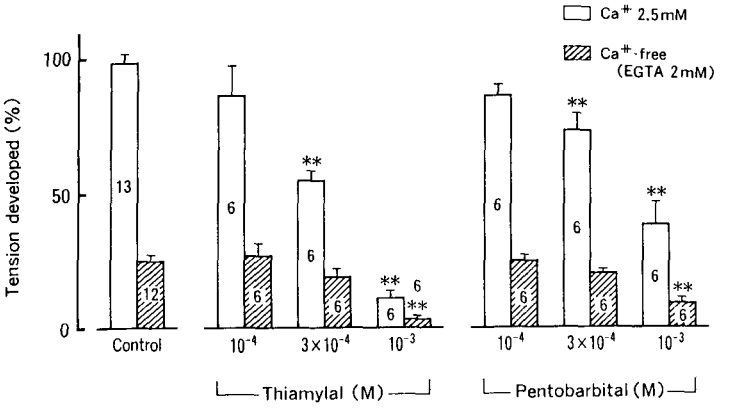

Fig. 3. Modification by thiamylal and pentobarbital of contraction induced by NE (10^{-6}M) in normal or Ca^{++}-free medium. Contraction was induced by 10^{-6}M NE in normal or Ca^{++}-free medium after 20 min treatment with thiamylal, pentobarbital (10^{-4} to 10^{-3}M) or an equivalent volume of distilled water (control). NE (10^{-6}M)-induced contraction in normal Ca^{++} medium before treatment was taken as 100%, the absolute values of which averaged 854 ± 58 mg (n = 14). Values in the bars indicate the numbers of strips studied. $*P < 0.05$, $**P < 0.01$ versus control.

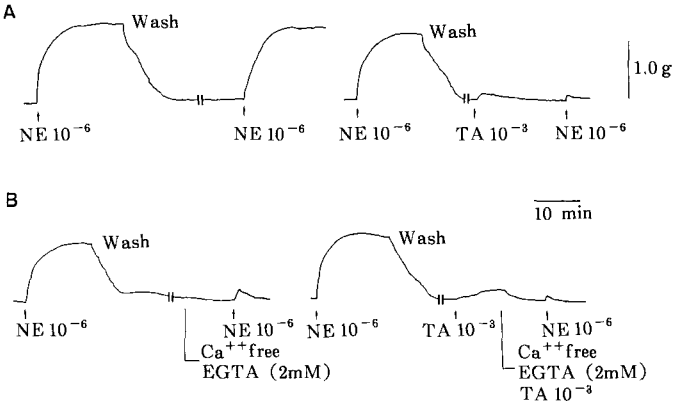

Fig. 4. Actual recordings of contractions induced by NE (10^{-6}M) in normal (A) or Ca^{++}-free (B) medium with or without thiamylal (10^{-3}M). TA; thiamylal, NE; norepinephrine, Wash; washing of the strips with normal medium (at least three times).

Drugs used were thiamylal sodium (Kyorin Pharmaceutical Co., Tokyo, Japan), pentobarbital, caffeine, norepinephrine (Nacalai Tesque, Kyoto, Japan), A23187 (Sigma), acetylcholine (Daiichi Pharmaceutical Co., Tokyo, Japan) and nifedipine (Bayer). Caffeine was dissolved in Ca^{++}-free Krebs' bicarbonate solution to a concentration of 10^{-2}M, and the bathing fluid was replaced with this solution for exposure to caffeine. NE was dissolved in 0.1% sodium pyrosulfate, and nifedipine in 10% ethanol. Other drugs were dissolved in distilled water, and added directly to the bathing medium; the volumes added were less than 100 μL.

Statistical analysis

Data were expressed as means ± SEM. The differences in Ca^{++}-induced contractions between control and thiamylal-treated or pentobarbital-treated preparations were analyzed by Student's t test for paired data. Other data were analyzed statistically by analysis of variance and Newman Keuls' multiple range test. Differences at P values of less than 0.05 were considered significant.

Results

KCl (20 mM)-induced contraction in normal medium (2.5 mM Ca^{++}) was inhibited significantly by thiamylal at a concentration of over 3×10^{-5}M and by pentobarbital at over 10^{-4}M (fig. 1). In aortic strips exposed to Ca^{++}-free medium containing 0.1

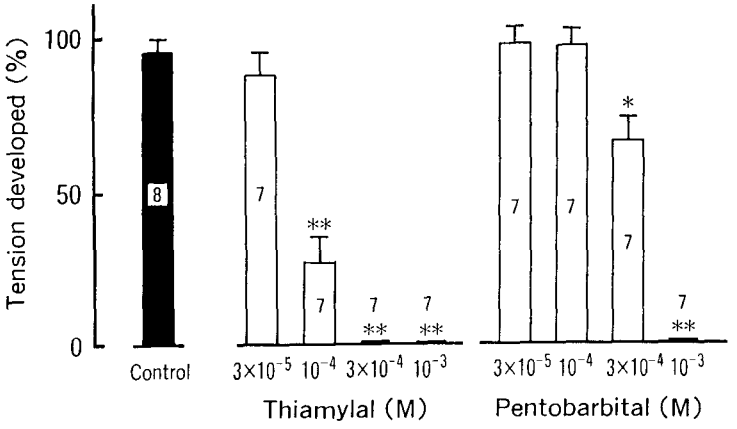

Fig. 5. Modification by thiamylal and pentobarbital of contraction induced by caffeine (10 mM). Contraction was induced by 10 mM caffeine after 20 min of Ca^{++} loading and 10 min of exposure to Ca^{++}-free medium. Thiamylal, pentobarbital (3×10^{-5} to 10^{-3}M) or an equivalent volume of distilled water (control) was added to the Ca^{++}-free medium (10 min treatment). Caffeine-induced contraction before treatment was taken as 100%, the absolute values of which averaged 58.5 ± 8.3 mg (n = 17). Values in the bars indicate the numbers of strips studied. $*P < 0.05$, $**P < 0.01$ versus control.

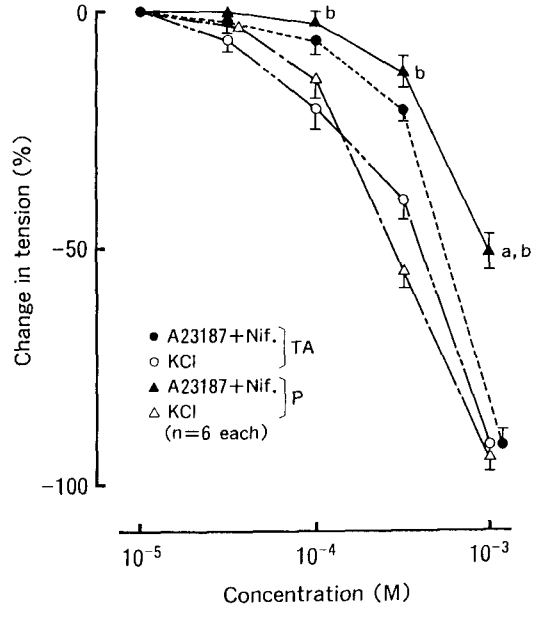

Fig. 6. Change in tension induced by thiamylal and pentobarbital (10^{-5} to 10^{-3}M) in endothelium-denuded aortic strips contracted with KCl (20 mM) and those which had been pretreated with nifedipine (5×10^{-7}M) and contracted with A23187. Maximum relaxation induced by papaverine (10^{-4}M) was taken as 100%, the absolute values of which averaged 467 ± 65 mg (n = 12) in KCl-contracted strips and 523 ± 104 mg (n = 12) in A23187-contracted strips. Nif.; nifedipine, TA; thiamylal, P; pentobarbital. a; $P < 0.01$ pentobarbital versus thiamylal, b; $P < 0.01$ KCl versus A23187.

mM EGTA, the addition of KCl did not produce any significant contraction, and reintroduction of Ca^{++} (0.5 to 10 mM) to the medium induced a dose-related contraction. As shown in figure 2, pretreatment of these strips with thiamylal at 10^{-4}M and 3×10^{-4}M shifted the dose-contraction response curve for Ca^{++} to the right. Thiamylal at 10^{-3}M and pentobarbital at 10^{-4} to 10^{-3}M shifted the curve downwards.

NE (10^{-6}M)-induced contraction in normal medium was inhibited by thiamylal and pentobarbital at concentrations over 3×10^{-4}M. In Ca^{++}-free medium containing 2 mM EGTA, NE induced a transient but significant contraction, which averaged 25.0 ± 1.3% (n = 12) of the contraction induced by NE in normal medium. This contraction in Ca^{++}-free medium was significantly inhibited by thiamylal and pentobarbital at a concentration of 10^{-3}M (fig. 3). The inhibition by thiamylal of NE-induced contraction in normal medium and Ca^{++}-free medium are shown in figure 4.

Caffeine (10 mM)-induced contraction was significantly inhibited by thiamylal at a concentration of over 10^{-4}M, and by pentobarbital at over 3×10^{-4}M (fig. 5); caffeine-induced contraction was significantly more susceptible to thiamylal than to pentobarbi-

tal.

KCl-precontracted strips were relaxed by thiamylal and pentobarbital at a concentration of 3×10^{-5} to 10^{-3}M; the relaxing effect of thiamylal did not differ significantly from that of pentobarbital in KCl-contracted strips. A23187-contracted strips were relaxed by thiamylal and pentobarbital within a concentration range of 3×10^{-4} to 10^{-3}M; the effect of thiamylal at 10^{-3}M was significantly greater than that of pentobarbital at the same concentration (fig. 6).

Discussion

Barbiturates have been shown to suppress the contraction of isolated vascular smooth muscle of various origins[1-11], and most investigators[3-7,9,10]have ascribed this effect to a Ca channel-blocking action[16]. However, in dog mesenteric artery, thiamylal has been revealed to suppress contraction through intracellular actions[8,11]. The present study compared the effects of thiamylal and pentobarbital in clinically relevant concentrations[17,18] on contractions mediated through various mechanisms in rat aorta, in an attempt to elucidate the mechanism of the vascular effects of these two barbiturates.

It was demonstrated that KCl-induced contraction of rat aorta was inhibited by thiamylal and pentobarbital to the same extent as shown in figure 1. KCl is known to induce depolarization of vascular smooth muscle cells, associated with opening of voltage-dependent Ca^{++} channels and subsequently with influx of Ca^{++} from the extracellular fluid[19]. Altura et al. using ^{45}Ca demonstrated that the uptake of extracellular Ca^{++} by vascular smooth muscle of rat aorta was inhibited by pentobarbital[16], and the present results may further support the hypothesis that pentobarbital and thiamylal have strong Ca^{++}-channel blocking actions.

In the present study, however, as shown in figure 2, differences were revealed in the modifications by thiamylal and pentobarbital of the contractile response to Ca^{++} of KCl-depolarized strips which had been soaked in Ca^{++}-free medium; the maximum response to Ca^{++} was inhibited by pentobarbital even at lower concentrations, but by thiamylal only at high concentrations. Our previous studies have shown that thiamylal itself has a vasoconstrictor effect which depends on an increase of Ca^{++} influx from the extracellular fluid, although pentobarbital lacks this constrictor effect[7,8,13]. Therefore, it is likely that the constrictor effect of thiamylal, which had not been expressed in Ca^{++}-free media, were expressed following addition of Ca^{++} to the media, and thus the constrictor effect of thiamylal were added to that induced by KCl at each Ca^{++} concentration, thereby, shifting the dose-contraction response curves for Ca^{++} upwards in comparison with pentobarbital.

The present study also revealed that thiamylal and pentobarbital inhibit contractions induced by a Ca ionophore in the presence of a Ca-channel blocker, and those by caffeine and by NE in Ca^{++}-free medium, which are obviously not mediated through Ca^{++} influx from the extracellular fluid. Ca ionophores are known to increase the concentration of intracellular free Ca^{++} by increasing the Ca^{++} permeability of the intracellular organelles which store Ca^{++} such as sarcoplasmic reticulum and the permeability of the plasma membrane[17]. In the present study, a Ca ionophore (A23187, 5×10^{-6}M) caused sustained contraction of endothelium-denuded strips pretreated with a high concentration (5×10^{-7}M) of nifedipine, and these strips were not relaxed by increasing the concentration of nifedipine or addition of other Ca-channel blockers, indicating that the Ca channels sensitive to nifedipine were inactivated. However, the strips were relaxed by higher concentrations of thiamylal and pentobarbital, although the effect of pentobarbital was significantly less potent than that of thiamylal. Caffeine is known to release intracellularly stored Ca^{++} from the sarcoplasmic reticulum[20,21], and the present study showed that thiamylal strongly inhibited contraction of the strips when it was applied during the Ca^{++}-release phase induced by caffeine, in agreement with previous findings in dog mesenteric artery[11]. On the other hand, only high concentrations

of pentobarbital affected the caffeine-induced contraction. Thus it was indicated that these two barbiturates have an intracellular action that induces relaxation. This action may involve an inhibitory effect on the intracellular contractile machinery including regulatory proteins or calmodulin[22]. Furthermore, the potencies of the intracellular actions of thiamylal and pentobarbital in vascular smooth muscle were shown to differ significantly; thiamylal appeared to have a potent intracellular action in addition to a potent Ca^{++} channel-blocking action, whereas pentobarbital appeared to act mainly on the Ca^{++} channels of the plasma membrane.

In spite of these findings, which suggest a strong, non-specific depressor effect of thiamylal in vascular smooth muscle, the contraction induced by NE (10^{-6}M) in normal Ca^{++} medium was less susceptible to inhibition by thiamylal than KCl-induced contraction, and the contraction induced by NE in Ca^{++}-free medium was also less susceptible to thiamylal than the caffeine-induced contraction. NE is known to induce contraction of vascular smooth muscle by binding to receptors, thus opening receptor-operated Ca^{++} channels in the plasma membrane, and releasing inositol-1,4,5 triphosphate, which in turn releases Ca^{++} from intracellular storage sites[23]. Studies by Fukuda et al.[24]using rabbit pulmonary artery, and by ourselves[7] using rat aorta have shown that thiobarbiturates potentiate the contractile response to lower concentrations of alpha-adrenergic agonists. Therefore, it may be hypothesized that thiamylal has an ability to enhance the cellular events induced by NE (even at higher concentrations), and that this specific potentiating effect counteracts the intracellular depressor effect or Ca^{++} channel-blocking action of thiamylal.

In summary, it has been demonstrated that in vascular smooth muscle of rat aorta, thiamylal exerts an intracellular as well as Ca channel-blocking action, and that the intracellular action of pentobarbital is significantly less potent than that of thiamylal.

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