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Alpha-adrenergic Blocking Action of Fentanyl on the Isolated Aorta of the Rabbit

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The contractile response of helically-cut strips of rabbit ascending aorta to transmural electrical stimulation was attenuated in a dose-dependent manner by treatment for 20 min with fentanyl, 10^{-6} to 10^{-5} M. Fentanyl also shifted the dose–response curve of the contractile response of aorta to norepinephrine to the right. The response to transmural stimulation was more resistant to fentanyl than was the response to an equipotent dose of norepinephrine. The inhibitory effect of fentanyl was neither prevented nor reversed by naloxone, but was partially reversed by repeated washing of the preparations. The contractile responses to histamine and serotonin were not significantly altered by fentanyl. Treatment with fentanyl as well as phentolamine protected alpha-adrenergic receptors from persistent blockade by phenoxybenzamine. Morphine to 10^{-3} M failed to influence the dose–response curve of norepinephrine significantly. It may be concluded that fentanyl reversibly blocks alpha-adrenergic receptors in a competitive manner in vascular smooth muscle, and the potency of fentanyl is approximately 1/30 that of phentolamine. (Key words: Analgesics, narcotic, fentanyl; Analgesics, narcotic, morphine; Anesthetics, intravenous, fentanyl; Anesthetics, intravenous, morphine; Sympathetic nervous system, alpha-adrenergic blockade; Sympathetic nervous system, norepinephrine.)

FENTANYL given intravenously in clinical doses (4.5 to 10 μg/kg body weight) decreases systemic blood pressure slightly,[1,2] while at higher doses (20 to 160 μg/kg body weight) it causes significant decreases in blood pressure and peripheral vascular resistance.[3–5] These circulatory changes are not abolished by pretreatment with antihistamic and anticholinergic drugs.[4] Little information is available concerning the effects of fentanyl on sympathetic control of vascular smooth muscle. The present study was undertaken to examine the effects of fentanyl on the responses of isolated rabbit aorta to stimulation of sympathetic nerves and norepinephrine administration. Morphine was used as a comparative drug.

Methods

Albino rabbits of both sexes, weighing 2.0 to 3.0 kg, were sacrificed by bleeding from common carotid arteries during ether anesthesia. The ascending and thoracic aorta down to the level of the diaphragm was rapidly removed and cut helically into strips approximately 25 mm long. These strips were fixed vertically between hooks in a 20-ml muscle bath containing a nutrient solution, comprised of (mM): sodium, 162.1; potassium 5.4; calcium, 2.2, chloride, 157.0; bicarbonate, 14.9; dextrose, 5.6. The upper end of the strips was connected to the lever of a force transducer and the resting tension was adjusted to 2.0 g. These manipulations were completed within a 20-min period. The bathing medium was bubbled with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide and was maintained at 37.0 ± 0.5 C. The *p*H of the solution was 7.2 to 7.3. Before the start of experiments, the preparations were allowed to equilibrate for 90 to 120 min, during which time the solution was replaced every 15 to 20 min.

For studies of the responsiveness to transmural electrical stimulation, the ascending aortic strips were placed between a pair of platinum stimulating electrodes, approximately 2 mm apart. The gaps between the electrodes and the strip were wide enough to allow for undisturbed vascular contraction and yet sufficiently narrow to permit effective stimulation of intramural nerve terminals.[6] The preparations were transmurally stimulated by a train of square pulses. Supramaximal stimuli (approximately 80 V[7]) of 0.3-msec duration were delivered at frequencies of 20, 5 or 2/sec, each train of stimuli consisting of a total of 200 pulses. Transmural stimulation was applied repeatedly at a frequency of 20/sec until steady responses at this frequency were attained; the frequency–response relationship was then obtained. Stimulus pulses were provided by an electronic stimulator.‡ Aortic contractions induced by stimulation for 10 sec at a frequency of 20/sec before the addition of blocking agents were used as the control values.

Norepinephrine, histamine and serotonin were applied directly to the bathing medium in cumulative concentrations. The tensions developed by norepinephrine, 5×10^{-5} M, histamine, 2×10^{-4} M, and serotonin, 10^{-5} M in medium free of blocking agents were taken as 100 per cent. The dose–response relationships of norepinephrine, histamine, or serotonin and the contractile responses to transmural stimulation were obtained after a 20-min exposure of preparations to the test drugs. Drugs studied were *dl*-norepinephrine hydrochloride, histamine dihydrochloride, serotonin creatinine sulfate, *dl*-phenoxybenzamine hydrochloride,

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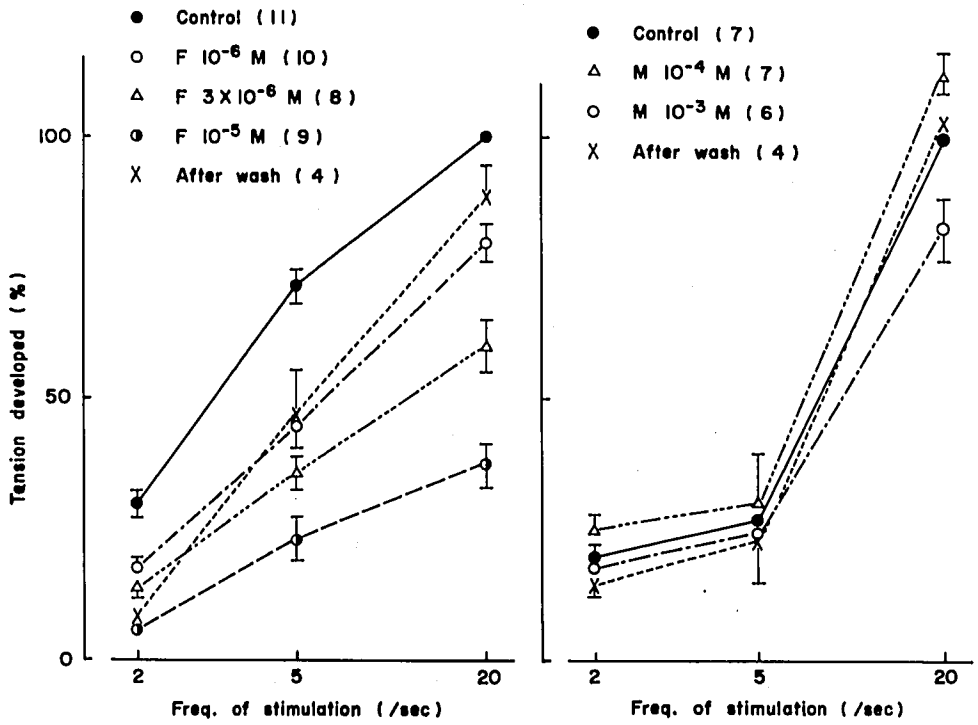

FIG. 1. Modification by fentanyl and morphine of the contractile response of ascending aorta to transmural electrical stimulation. The response at a frequency of 20/sec in control medium was taken as 100 per cent; mean values of contraction in experiments with fentanyl and morphine were 0.68 ± 0.09 g (N = 11) and 0.69 ± 0.09 g (N = 7), respectively. F, fentanyl; M, morphine; after wash, after repeated washing of preparations soaked in fentanyl, 10^{-5} M, or morphine, 10^{-3} M. Figures in parentheses indicate the numbers of preparations used.

phentolamine mesylate, fentanyl citrate, morphine hydrochloride, naloxone hydrochloride, bretylium tosylate, and tetrodotoxin. Aortic contractions were displayed on an ink-writing oscillograph.§

For the study of interactions between fentanyl and phenoxybenzamine, the dose–response relationship of norepinephrine was obtained first. Preparations were repeatedly washed and equilibrated for 40 to 50 min. In control experiments (shown as "nontreated" in fig. 6), preparations were left for 30 min in the medium without any blocking agent but were treated for another 30 min with phenoxybenzamine, 5×10^{-7} M, alone. In test experiments, preparations were pretreated for 30 min with either fentanyl or phentolamine and for another 30 min with phenoxybenzamine. After 30 min of exposure to phenoxybenzamine, preparations were repeatedly washed with fresh fluids and equilibrated. The dose–response relationships of norepinephrine were then obtained and compared with those obtained before the treatment with blocking agents.

The results are expressed as mean values and standard errors of the means. Comparisons were made using Student's t test.

§ Sanei Sokki Company, Tokyo.

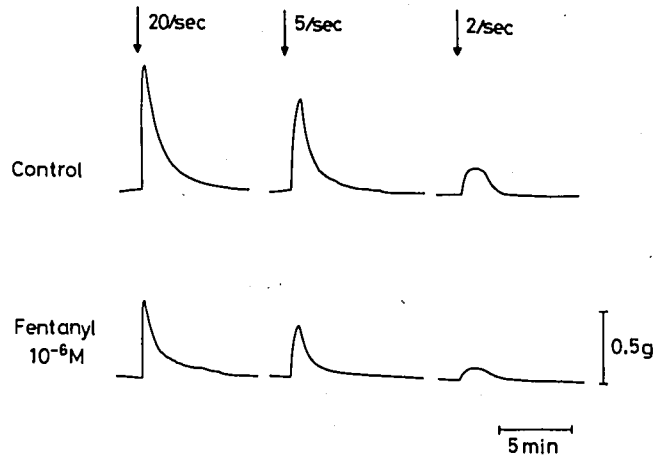

FIG. 2. Fentanyl-induced inhibition in the response of an ascending aortic strip to transmural electrical stimulation. Stimulation applied: 20/sec for 10 sec., 5/sec for 40 sec, and 2/sec for 100 sec.

Results

Effects of Fentanyl on the Contractile Response to Transmural Stimulation

Transmural electrical stimulation of aorta produced a transient, frequency-dependent contraction, which could be abolished by pretreatment with tetrodotoxin, 10^{-7} M, phentolamine, 10^{-6} M, and bretylium, 2×10^{-5} M, in four preparations studied. The addition of fentanyl in concentrations of 10^{-6}, 3×10^{-6}, and 10^{-5} M failed to produce any alteration in the aortic tension. Treatment for 20 min with these concentrations of fentanyl caused a significant decrease in the tension developed by transmural electrical stimulation in a dose-dependent manner (fig. 1). A typical recording of the inhibitory effect of fentanyl is shown in figure 2. The fentanyl-induced inhibition was neither prevented nor reversed by naloxone, 10^{-6}

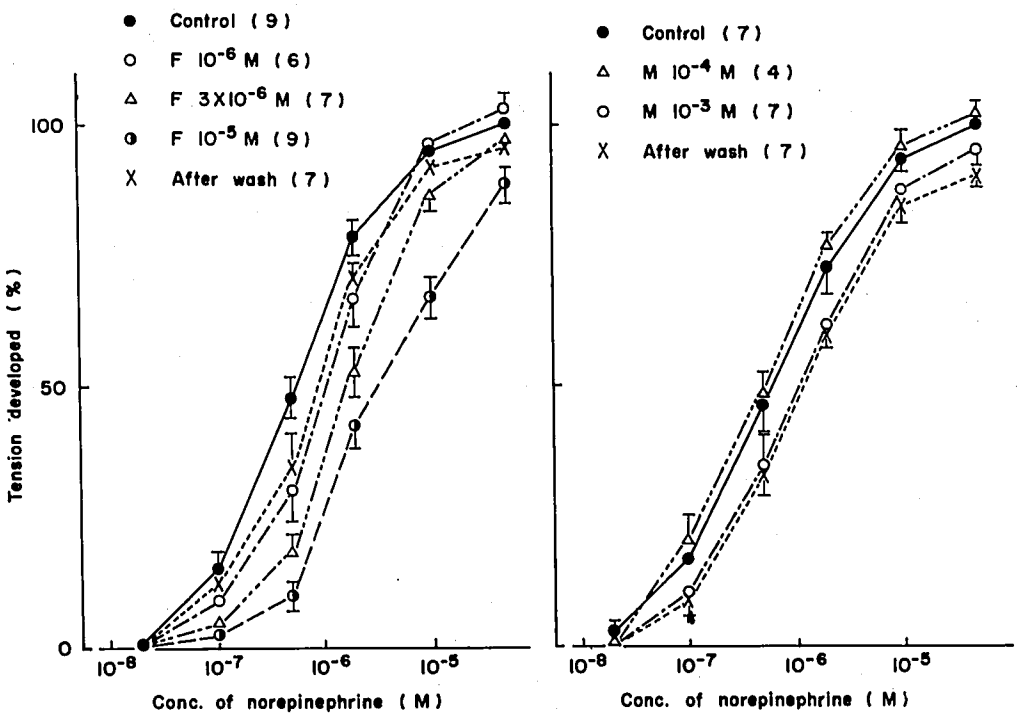

FIG. 3. Alterations in the dose–response relationship of norepinephrine induced by fentanyl and morphine. The response to norepinephrine, 5×10^{-5} M, in control medium was taken as 100 per cent; mean values of contraction in experiments with fentanyl and morphine were 3.46 ± 0.32 g (N = 9) and 2.76 ± 0.19 g (N = 7), respectively.

and 10^{-5} M, but was partially reversed by repeated replacement of fentanyl-containing medium with fresh fluids.

The mean concentration of exogenous norepinephrine necessary to cause the same magnitude of contractions as that induced by transmural stimulation at a frequency of 20/sec was 1.2×10^{-7} M. Inhibition of the response to this concentration of norepinephrine by fentanyl was always greater than that with transmural stimulation at 20/sec; mean values of the inhibition induced by fentanyl 10^{-6}, 3×10^{-6}, and 10^{-5} M were 39.3 ± 3.5 per cent (N = 6), 66.9 ± 2.7 per cent (N = 7), and 81.3 ± 1.8 per cent (N = 9), respectively, for norepinephrine-induced contraction and 19.7 ± 3.2 per cent (N = 10), 40.4 ± 5.9 per cent (N = 8), and 63.0 ± 6.2 per cent (N = 9), respectively, for contraction induced by the neural stimulation.

In contrast to fentanyl, morphine, 10^{-4} M, caused a significant increase ($P < 0.01$) in the tension developed by transmural stimulation at a frequency of 20/sec but not at frequencies of 5 and 2/sec (fig. 1). Further increase in the concentration of morphine to 10^{-3} M significantly decreased the response to transmural stimulation at 20/sec ($P < 0.01$). Neither the potentiation nor the inhibition by morphine was prevented by treatment with naloxone, 10^{-6} and 10^{-5} M.

EFFECTS OF FENTANYL ON RESPONSES TO NOREPINEPHRINE, HISTAMINE AND SEROTONIN

The addition of norepinephrine in concentrations ranging from 2×10^{-8} to 5×10^{-5} M caused dose-related contraction in strips of thoracic aorta. Pretreatment with fentanyl, 10^{-6}, 3×10^{-6}, and 10^{-5} M, shifted the dose–response curve for norepinephrine to the right in a dose-dependent manner (fig. 3). This inhibitory effect was not reversed by naloxone, 10^{-5} M, but was partially reversed by repeated washing of the preparations.

Inhibitory effects of fentanyl and phentolamine on norepinephrine-induced contraction were compared. Plot of norepinephrine dose ratios against log concentrations of fentanyl and phentolamine gave straight lines with slopes of −0.76 and −0.99,

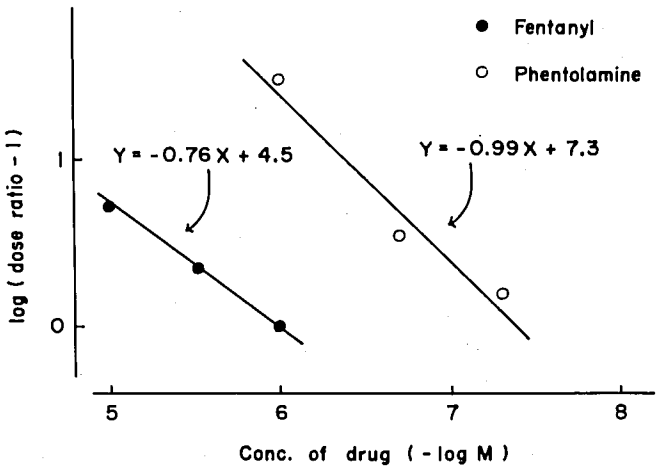

FIG. 4. Relationship between dose ratios of norepinephrine and log concentrations of fentanyl or phentolamine. The dose ratio was obtained from dose–response curves of norepinephrine in the thoracic aorta as the ratio of median effective concentration in medium containing blocking agents to the concentration in control medium.

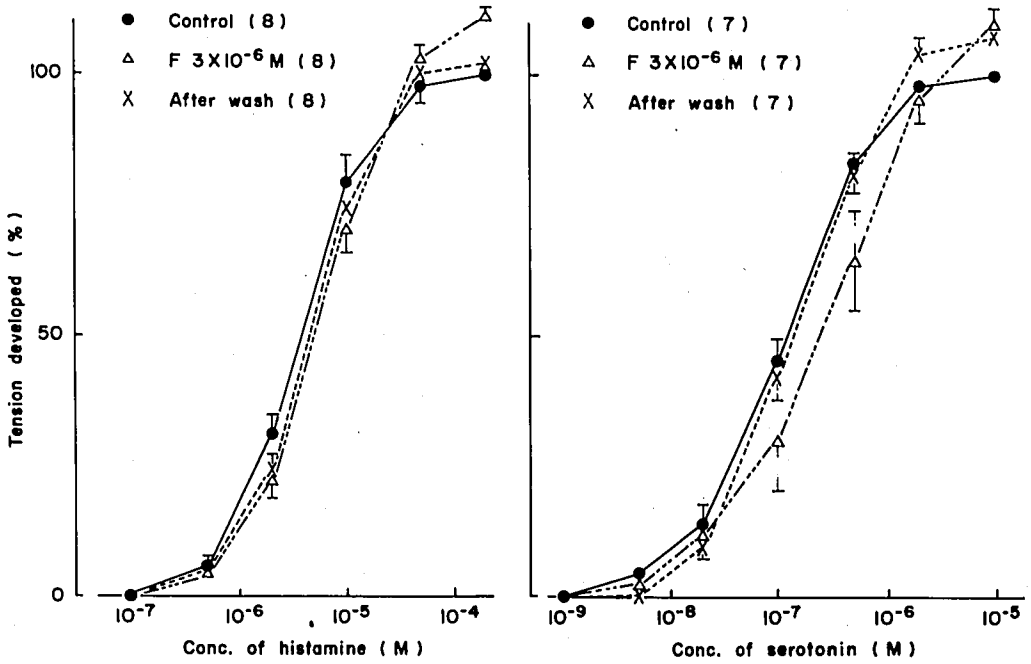

FIG. 5. Dose–response curves of histamine and serotonin in the presence and absence of fentanyl, 3×10^{-6} M. Contractions induced by histamine, 2×10^{-4} M, and serotonin, 10^{-5} M, were taken as 100 per cent; mean values of the maximum contraction induced by histamine and serotonin in control medium were 2.72 ± 0.11 g (N = 8) and 2.56 ± 0.20 g (N = 7), respectively.

respectively (fig. 4). Mean pA_2 values of these agents obtained in this figure were 5.9 and 7.4, respectively; thus fentanyl was 1/32 as potent as phentolamine. The pA_2 value represents the negative logarithm to base of 10 of the molar concentration of an antagonist (fentanyl or phentolamine in this study) that causes a doubling of the concentration of an agonist (norepinephrine) to compensate for the action of the antagonist,[8,9] and is obtained as a cross point of the horizontal line at zero on the ordinate and the regression line in figure 4.

Fentanyl, 3×10^{-6} M, a concentration sufficient to decrease the contractile responses to transmural stimulation and norepinephrine significantly, failed to alter the dose–response curves of histamine and serotonin (fig. 5).

Treatment with morphine, 10^{-4} and 10^{-3} M, did not significantly influence the contractile response to norepinephrine, although trends toward a decrease in the response were observed at 10^{-3} M (fig. 3).

INTERACTION BETWEEN FENTANYL AND PHENOXYBENZAMINE

Treatment with phenoxybenzamine suppressed the contractile response of thoracic aortic strips to norepinephrine (fig. 6). Fentanyl prevented the blocking action of phenoxybenzamine in a dose-dependent manner. Phentolamine was approximately 30 times more effective than fentanyl in preventing the receptor blocking action of phenoxybenzamine.

Discussion

The contractile response to transmural electrical stimulation applied under experimental conditions in the present study is considered to result from norepinephrine released from adrenergic nerve terminals innervating the vascular wall, since the response is abolished by tetrodotoxin, alpha-adrenergic receptor blocking agents, and adrenergic neuron blocking agents.[7,10,11] Treatment with fentanyl significantly attenuated the contractions of rabbit aortic strips induced by the transmural neural stimulation and shifted the dose–response curve of norepinephrine parallel to the right. Nonselective inhibition in the contractile response by fentanyl was excluded by the findings that the contractions induced by histamine and serotonin were not altered by fentanyl. Further, treatment with fentanyl effectively protected alpha-adrenergic receptors from the persistent blockade by phenoxybenzamine, as did phentolamine, strongly suggesting that fentanyl and phenoxybenzamine share the same receptive site.[12] Only blocking agents of a competitive type are effective in such a receptor protection.[9] From these findings, it may be concluded that fentanyl reversibly blocks alpha-adrenergic receptors in a competitive manner in vascular smooth muscles. Alpha blocking potency of fentanyl was approximately 1/30 that of phentolamine, so far as pA_2 values and protective potencies against phenoxybenzamine are concerned. Such an alpha blocking action of fentanyl was neither prevented nor reversed by naloxone, suggesting that this blockade is not related to its analgesic action or effects on respiration.

Contractions induced by transmural neural stimulation were more resistant to fentanyl than was the response to an equipotent dose of exogenous norepinephrine. This is consistent with the results obtained with known alpha blocking agents, phento-

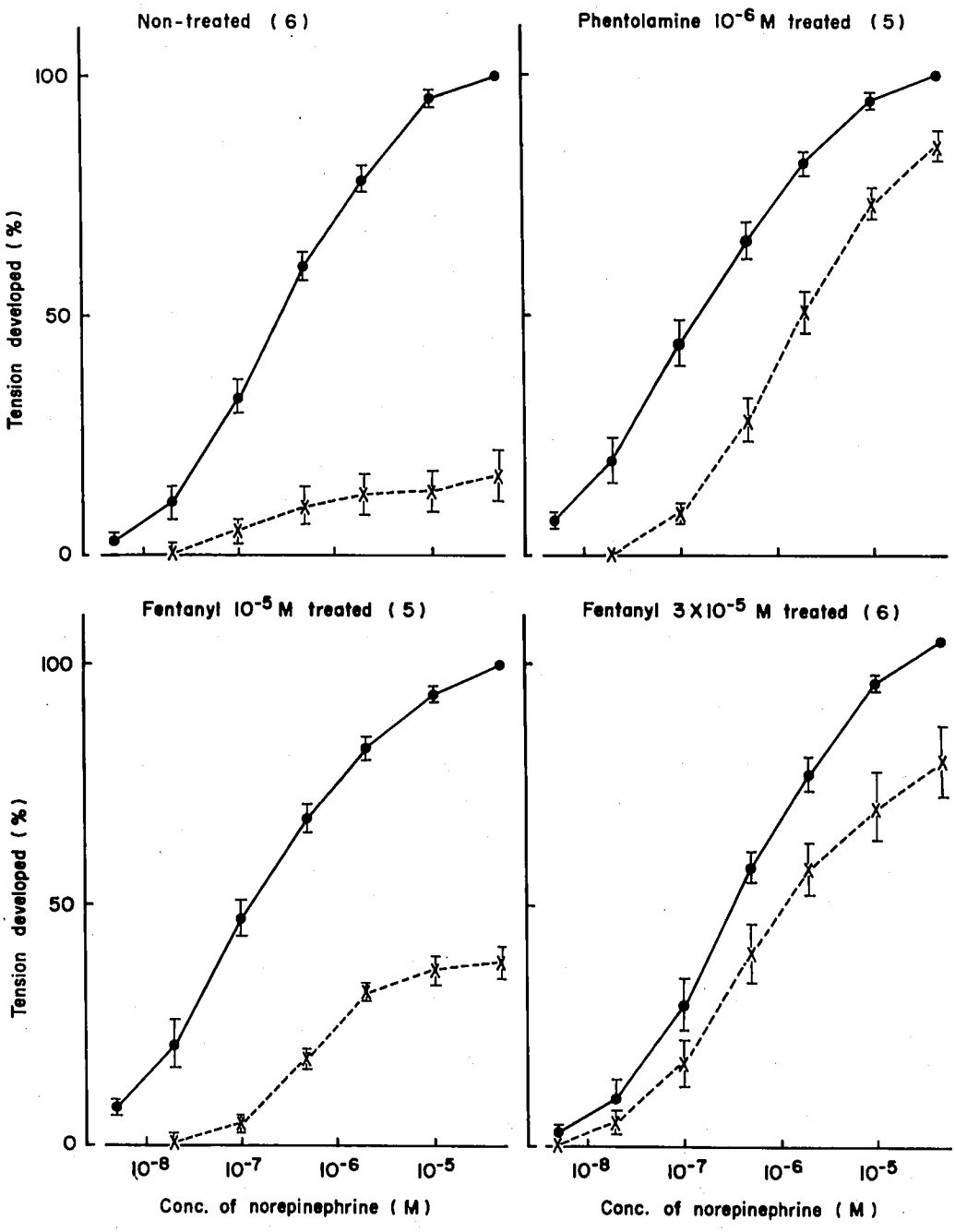

FIG. 6. Modification by treatment with fentanyl or phentolamine of phenoxybenzamine-induced alpha blockade in isolated thoracic aorta. *Solid lines*: before addition of phenoxybenzamine; *broken lines*: after washing of preparations soaked in phenoxybenzamine, 5×10^{-7} M. Contractions induced by norepinephrine, 5×10^{-5} M, in control medium were taken as 100 per cent; mean values of contraction in nontreated, fentanyl (10^{-5} and 3×10^{-5} M)-treated, and phentolamine - treated preparations were 2.78 ± 0.33 g (N = 6), 3.34 ± 0.31 g (N = 5), 2.40 ± 0.19 g (N = 6), and 3.69 ± 0.27 g (N = 5), respectively. Nontreated, treatment for 30 min with phenoxybenzamine alone; fentanyl- or phentolamine-treated, treatment for 30 min with fentanyl or phentolamine and for another 30 min with phenoxybenzamine. For further explanation, see text.

lamine, phenoxybenzamine, and yohimbine.[13,14] Bevan and Su[14] postulated that a uniform distribution of exogenous and a non-uniform (high concentration close to nerve terminals, the further the distance from the nerves, the less is the concentration of norepinephrine) distribution of neurogenic norepinephrine exist throughout the medium. Therefore, when equal responses to neurogenic and exogenous norepinephrine are induced, the peak concentration for the neurogenic amine must be higher than that for the exogenous amine. The concentration of an alpha-receptor blocking agent is obviously a function of norepinephrine concentration at the receptor; the higher the concentration of norepinephrine, the higher the concentration of the blocking agent. This may explain the different susceptibilities of actions of neurogenic and exogenous norepinephrine to fentanyl, such as may occur with phentolamine and phenoxybenzamine.

Fentanyl citrate (molecular weight 528) is clinically used as an analgesic at an intravenous dose of 0.05 mg/10 kg body weight. If this drug is distributed uniformly in circulating blood (roughly 100 ml/kg body weight), without taking into consideration excretion or metabolism, the predicted concentration of fentanyl will be approximately

5×10^{-8} g/ml, or 10^{-7} M. This is approximately 1/10 the concentration needed to attenuate the response of isolated aorta to the alpha-adrenergic receptor stimulant, norepinephrine, significantly. The *in-vitro* aortic strip used in the present study may be less sensitive to the blocking agent than the *in-vivo* microvascular smooth muscle; therefore it is possible that fentanyl in a clinical dose exerts some alpha-blocking action in human vasculature. Further, it has been demonstrated that fentanyl administered intravenously is accumulated in the brain, the concentration ratio in the plasma and the brain being approximately 1:10.[15] These findings suggest that fentanyl may exert an alpha-adrenergic blocking action on the central nervous system.

Treatment with morphine, 10^{-4} M, potentiated the response to transmural neural stimulation at 20/sec. This may derive from impaired uptake of norepinephrine by sympathetic nerve terminals.[16] However, such potentiation by morphine was inconsistent when the preparations were stimulated at lower frequencies of 5 and 2/sec. At 10^{-3} M, morphine tended to decrease the response to transmural stimulation but did not alter the response to exogenous norepinephrine. Therefore, the alpha-adrenergic blocking action of morphine even in such a high concentration is, if any, only slight.

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