Anesthesiology
57:381–388, 1982

Effects of Furosemide on the Neuromuscular Junction

K. A. Scappaticci, Ph.D., J. A. Ham, M.D.,† Y. J. Sohn, M.D.,‡ R. D. Miller, M.D.,‡ K. L. Dretchen, Ph.D.§*

These studies investigated the direct effects of furosemide on neuromuscular transmission using the *in vitro* rat phrenic nerve diaphragm and the *in vivo* cat soleus nerve muscle preparations. Furosemide (10^{-6}–10^{-4} M) reduced the concentration of *d*-tubocurarine required to achieve 50% twitch tension depression in the indirectly stimulated rat diaphragm. Intrarterial injection of furosemide had a biphasic effect on the cat neuromuscular junction. At low doses (0.1–10.0 μg/kg) the drug had a depressant effect, reduced the force of muscle contraction, prevented nerve and muscle responses to NaF and dibutryl cyclic AMP, and intensified the neuromuscular blockade produced by *d*-tubocurarine and succinylcholine. In contrast, in higher doses (1–4 mg/kg) furosemide produced stimulus-bound repetitive neural activity, initiated neural activity, increased the force of muscle contraction, enhanced nerve and muscle responses to NaF and dibutyrl cyclic AMP, and antagonized *d*-tubocurarine and succinylcholine blockades. Furosemide had no effect on denervated preparations. High doses of furosemide inhibit noncompetitively both the high- and low-affinity forms of the enzyme cyclic AMP phosphodiesterase in both soluble and particulate fractions of cat sciatic nerve. Thus, furosemide has direct effects on neuromuscular transmission, but the direction of these effects is dose-dependent. (Key words: neuromuscular relaxants: succinylcholine; *d*-tubocurarine. Neuromuscular transmission. Pharmacology: furosemide.)

FUROSEMIDE, a frequently prescribed diuretic, augments the neuromuscular blockade induced by *d*-tubocurarine in humans.[1] It is possible that this effect of furosemide is an indirect consequence of its diuretic action, due for example, to alteration of extracellular electrolyte concentrations, or to a redistribution of *d*-tubocurarine that increases its effective serum concentration. However, previous work argues against such indirect actions. Reductions in extracellular potassium and calcium concentrations have been reported to augment *d*-tubocurarine-induced neuromuscular blockade,[1,2] but the neuromuscular response to furosemide is immediate, and furosemide does not acutely alter the plasma calcium and potassium concentrations.[3–5] Furthermore, Miller *et al.*[1] found no direct correlation between serum *d*-tubocurarine levels and the augmentation of *d*-tubocurarine-induced neuromuscular blockade following furosemide administration.

These considerations suggest that furosemide has a direct effect on the neuromuscular junction. The experiments presented here test this hypothesis in both *in vitro* (rat) and *in vivo* (cat) neuromuscular preparations.

Materials and Methods

In Vitro Rat Phrenic Nerve-Diaphragm Preparation

Nerve-diaphragms were dissected from male Sprague-Dawley rats (80–150 g) using the method of Bulbring.[6] The organ bath contained Tyrode's solution (mM): NaCl, 129; KCl, 4.0; $MgCl_2$, 0.5; $CaCl_2$, 2.7; NaH_2PO_4, 1.8; $NaHCO_3$, 20; and dextrose, 5.5 maintained at 36°C and bubbled with 95% O_2–5% CO_2. The phrenic nerve was stimulated supramaximally at 0.15 Hz, with a pulse duration of 0.3 ms. Twitch tension was monitored via a suture connecting the central tendon to a force-displacement transducer. An initial tension of 5 g was placed on the muscle. Twitch tensions were measured at different doses of furosemide alone (10^{-6} to 2×10^{-4} M), *d*-tubocurarine alone (0.1×10^{-7} to 2.0×10^{-7} M), and with a combination of the two drugs.

In Vivo Cat Soleus Nerve-Muscle Preparation

Cats (2.0–3.0 kg) were anesthetized with 70 mg/kg alpha-chloralose, iv. The surgical preparations have been described by Riker *et al.*[7] and Standaert.[8] All branches of the popliteal artery except that which supplies the soleus muscle were occluded, and all branches of the tibial nerve except that to the soleus muscle were severed. The tendon of the soleus muscle was attached to a strain gauge, a dorsal laminectomy was performed, the ventral root of L7 was cut, and a single functional axon to the soleus was placed over bipolar platinum recording electrodes. A bipolar platinum stimulating electrode was placed on the soleus nerve near its entry into the muscle. The soleus nerve was stimulated with supramaximal pulses (0.1-ms duration) applied once every 2.5 s. Tetanic stimulation consisted of a 10-s train of pulses at 400 Hz, applied every 5 min.

In some experiments, the soleus muscle was chronically denervated. Denervation was performed 14 days

* Postdoctoral Fellow, Department of Physiology/Biophysics, University of Miami.

† Research Associate, Departments of Anesthesia and Pharmacology, University of California.

‡ Associate Professor, Departments of Anesthesia and Pharmacology, University of California.

§ Associate Professor, Department of Pharmacology, Georgetown University.

Received from the Department of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, DC 20007, and the Department of Anesthesia and Pharmacology, University of California, San Francisco, California 94143. Accepted for publication April 26, 1982. Supported in part by a grant from the U. S. Public Health Service (GM-15571) and a grant from the National Science Foundation (BNS-78-26849).

Address reprint requests to Dr. Dretchen: Department of Pharmacology, Georgetown University, 3900 Reservoir Road, Washington, DC 20007.

0003-3022/82/1100/0381 $01.20

prior to recording by ligating and severing the sciatic nerve at the sciatic notch. Surgery was done using 25 mg/kg ketamine hydrochloride, im, as the anesthetic. During recording, the denervated muscle was stimulated directly via two 33-gauge stainless steel wires sewn in concentric rings 1 cm cranial to the musculotendinous junction. Denervated muscle was stimulated with supramaximal rectangular pulses (2-ms duration) applied once every 2.5 s.

Drug-initiated activity refers to drug-initiated bursts of action potentials that are not related to the external stimulus and occurs only in innervated muscles. Stimulus-bound repetitive activity refers to the neural activity following drug administration by which a single stimulus applied to the nerve evokes a train of action potentials, the first is the stimulus-evoked potential, the others are stimulus-bound repetitive activity.[9] Drug-initiated activity and stimulus-bound repetitive activity recorded extracelularly from the ventral root and muscle twitch tension were amplified and recorded simultaneously on a polygraph and on magnetic tape. The changes in the force of muscle contraction produced by drug administration or high-frequency stimulation of the nerve were expressed as the integral gram-seconds, the product of the increase in contraction strength times the time during which the contraction was above control.

All drugs were administered by rapid injection into the popliteal artery. Solutions were prepared by dissolving the compound in 0.85% NaCl solution so that 0.1 ml of solution contained the amount of material to be given per kilogram of animal weight. All solutions were adjusted to *p*H 7.4 with NaOH.

Cat Sciatic Nerve Cyclic AMP Phosphodiesterase

Preparation of Homogenate

Sciatic nerves from anesthetized cats were isolated from the region between the sciatic notch and the branch point of the common peroneal, sural, and tibial nerves and frozen for later use. On the day of assay, the nerve was thawed, desheathed, and weighed. The nerve was finely minced with scissors, suspended in buffer (160 mM Tris-Cl, 20 mM M_gCl_2, *p*H 8.0, 100 mg tissue/ml) and homogenized with 40–50 strokes from a motor-driven pestel in a glass-Teflon® homogenizer. The homogenate was centrifuged at 2000 × *g* for 5 min, and the resulting supernatant was centrifuged at 100,000 × *g* for 50 min. The high-speed supernatant was collected and diluted with homogenization buffer to a concentration of about 1 mg protein/ml. This supernatant fraction was used to determine the soluble activity of high-affinity cyclic AMP phosphodiesterase. The pellet fractions from the 2,000 and 100,000 × centrifugations were combined and resuspended in the original volume of homogenization buffer. This suspension was then centrifuged at 100,000 × *g* for 50 min, and the resulting washed pellet fraction resuspended in homogenization buffer to a concentration of approximately 2.5 mg protein/ml. This washed pellet fraction was used to determine the activity of high-affinity cyclic AMP phosphodiesterase. Low-affinity cyclic AMP phosphodiesterase activity (high cyclic AMP concentrations) was determined using a total homogenate prepared by filtering the crude homogenate through two layers of cotton gauze to remove any remaining connective tissue.

Assay of Phosphodiesterase Activity and Protein Determination

Phosphodiesterase activity was measured with ^{3}H-cyclic AMP as substrate using a slight modification of the two-step method of Thompson and Appleman.[10] Assay time (10–30 min) and protein concentration were adjusted to remain within the linear range of the assay in all cases. Protein was determined by the method of Lowry *et al.*[11] using bovine serum albumin as the standard. Enzyme activity was expressed as pmol cyclic AMP hydrolyzed per min per mg protein.

Chemicals

The following reagents were used: *d*-tubocurarine chloride, succinylcholine chloride, sodium fluoride, snake venom (*Ophiophagus hannah*) and theophylline, Sigma Chemical Co., St. Louis, Missouri; N^6, O^2-dibutyryl adenosine-3′:5′-cyclic monophosphate and adenosine-3′:5′-cyclic monophosphate, Boehringer Mannheim, Indianapolis, Indiana; [8-^{3}H]3′:5′-cAMP (specific activity 34.4 Ci/mmole) and [8-^{14}C] adenosine (specific activity 54.7 mCi/mmole) New England Nuclear, Boston, Massachusetts; anion exchange resin Dowex AG1-X2, 200–400 mesh, Bio-Rad Laboratories, Richmond, California; Liquiscint, National Diagnostic, Parsippany, New Jersey. Furosemide was a gift from Hoechst-Roussel Pharmaceuticals Inc., Somerville, New Jersey. All drugs and reagents were pure and preservative-free.

Statistical Analysis

Comparison of two sample means such as the response in the presence and absence of a single dose of furosemide was done using Student's *t* test, paired experiments. Multiple comparisons were made using an analysis of variance with Duncan's New Multiple Range test used for orthogonal contrasts. Best-fitting lines were calculated by the method of least-squares. In all cases, the data was reported as the means ± SE, and a P value of < 0.05 was considered significant.

Results

Effects of Furosemide on the *In Vitro* Rat Phrenic Nerve-Diaphragm Preparation

Furosemide alone (10^{-6} to 2×10^{-4}) had no effect on the indirectly evoked twitch tension. However, furosemide significantly reduced the concentration of *d*-tubocurarine required to achieve 50% depression (Ed_{50}). This enhancement of *d*-tubocurarine's blocking effect, increased with increasing furosemide concentration (table 1); 2.4×10^{-6} M furosemide lowered the ED_{50} by 50%, and 1.2×10^{-4} M lowered the ED_{50} by 90%.

TABLE 1. Effect of Furosemide on *d*-Tubocurarine Neuromuscular Blockade *In Vitro* Rat Phrenic Nerve-diaphragm Preparation

Concentration Furosemide in Bath	Concentration of *d*Tc Needed to Produce a 50% Depression of Twitch
——	$2.0 \pm 0.15 \pm 10^{-7}$ M* (0.136 ± 0.01 μg/ml)
2.4×10^{-6} M (8 μg/ml)	$1.0 \pm 0.15 \times 10^{-7}$ M† (0.07 ± 0.01 μg/ml)
1.2×10^{-4} M (400 μg/ml)	$0.22 \pm 0.03 \times 10^{-7}$ M† (0.015 ± 0.002 μg/ml)

* Mean ± SD, n = 6.
† Statistically significant dose-dependent shift of *d*Tc, $P < 0.05$.
The motor nerve was stimulated supramaximally for 0.3 ms at 0.15 Hz.

Effects of Furosemide on the *In Vivo* Cat Soleus Nerve Muscle Preparation

Effects of Furosemide at Low Doses

Intra-arterial injection of 0.1–100 μg/kg furosemide alone slightly inhibited neuromuscular transmission. This effect was maximal at a dose of 0.1 μg/kg, which reduced twitch tension to 92 ± 2% of control (n = 8, $P < 0.05$). The inhibition was maximal within 10 s after the injection and lasted from 15 to 40 s. Larger doses (1.0 and 10.0 μg/kg) did not produce greater inhibition or longer lasting effects. Doses exceeding 100 μg/kg did not alter twitch tension. Furosemide (0.1 to 1,000 μg/kg) had no consistent effect on post-tetanic stimulus-bound repetitive activity initiated in the nerve by high-frequency stimulation (400 Hz, 10 s).

The interactions between furosemide and *d*-tubocurarine or succinylcholine were examined by giving an intra-arterial injection of *d*-tubocurarine or succinylcholine sufficient to depress twitch tension approximately 75%, and then injecting furosemide intra-arterially after partial recovery (to less than 50% of control). Furosemide in doses of 0.1 and 10.0 μg/kg augmented *d*-tubocurarine's neuromuscular blockade. The lowest dose of furosemide (0.1 μg/kg) enhanced *d*-tubocurarine blockade to 65 ± 2% of control (n = 3). As shown

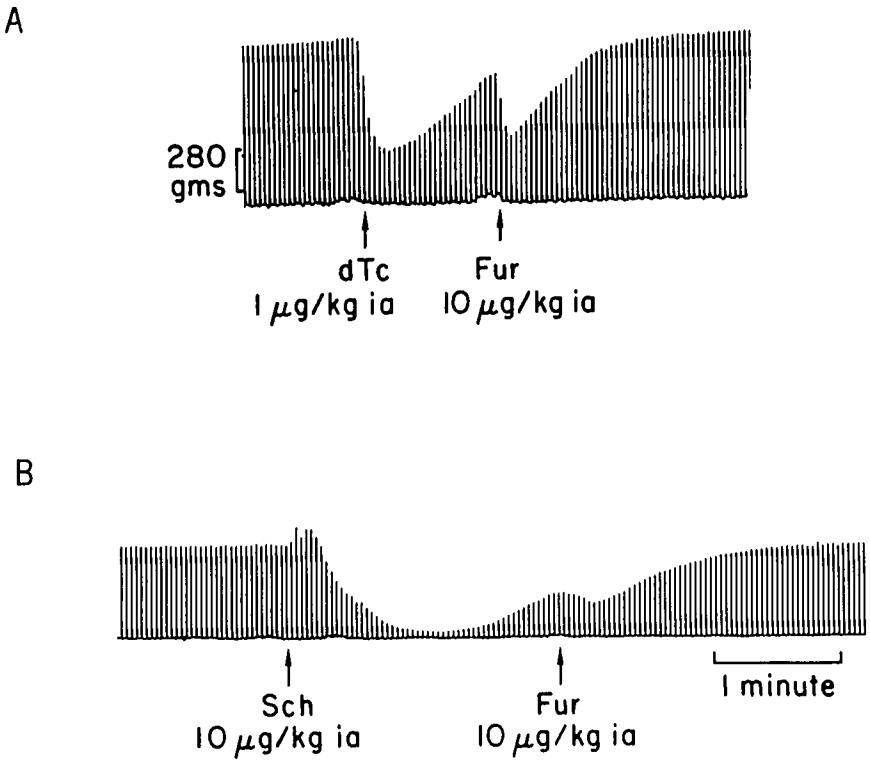

FIG. 1. Effect of low doses of furosemide (Fur) on *d*-tubocurarine (*d*Tc) and succinylcholine (Sch) neuromuscular blockade in the *in vivo* cat soleus muscle. Vertical scale denotes twitch tension evoked by nerve stimulation. Arrows indicate the intra-arterial administration of the indicated dose of each drug.

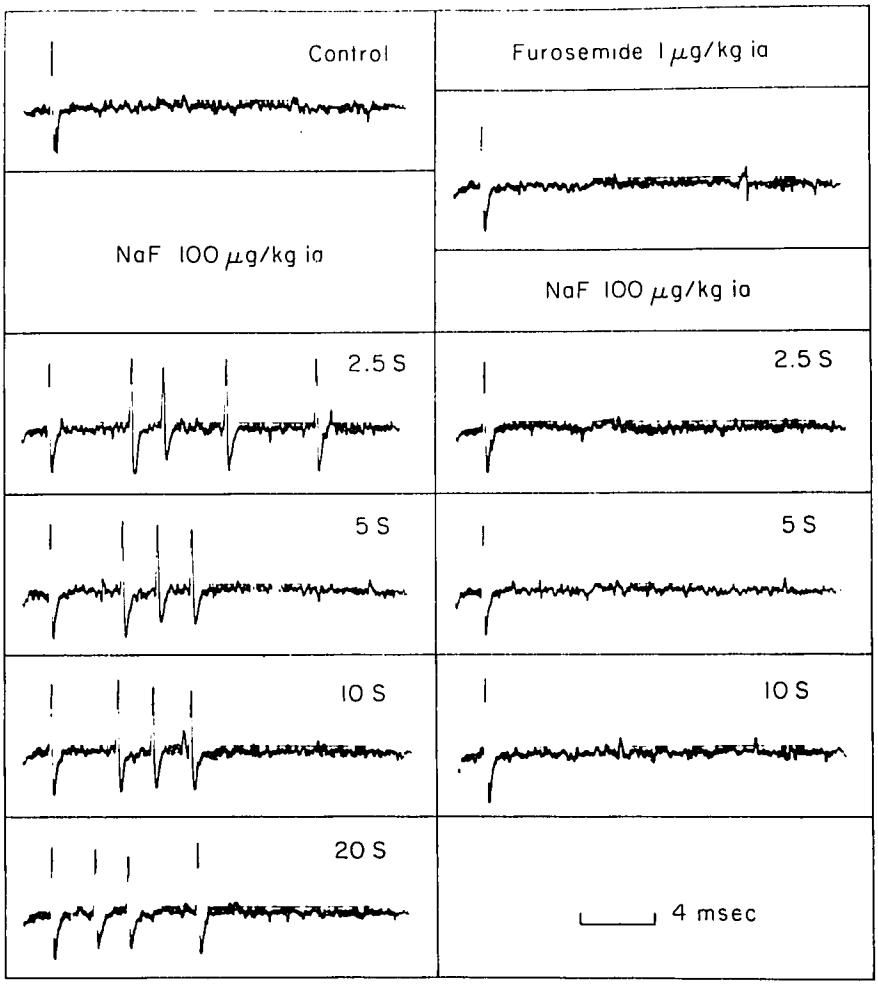

FIG. 2. Effect of low dose of furosemide on stimulus-bound repetitive activity produced in a soleus motor axon by NaF. The first trace on the left shows the response recorded in the ventral root following a single stimulus applied before either drug; subsequent traces show the responses to single stimuli applied at the times indicated after intra-arterial administration of NaF (100 μg/kg). The first trace on the right shows the ventral root response to a single stimulus applied after the administration of furosemide (1 μg/kg). Subsequent traces show responses to single stimuli when NaF was applied following furosemide administration.

in figure 1*A*, a hundred-fold higher dose of furosemide (10.0 μg/kg) produced a similar degree of depression to 59 ± 5% of control (n = 4). Furosemide also augmented the neuromuscular blockade produced by succinylcholine; 1.0 μg/kg furosemide enhanced succinylcholine blockade to 68 ± 12% of control (n = 6), and 10 μg/kg furosemide produced a similar degree of depression to 77 ± 9% of control (n = 6, fig. 1*B*).

The interaction between furosemide and NaF (an activator of adenylate cyclase) or dibutyryl cyclic AMP (a lipid-soluble cyclic AMP derivative) was studied by injecting furosemide (0.1 to 100 μg/kg) 2 min before an injection of NaF or dibutyryl cyclic AMP. Injection of NaF or dibutyryl cyclic AMP alone increases the muscle contraction evoked by a single nerve stimulus, due in part to the fact that these drugs induce stimulus-bound repetitive activity in the motor nerve (fig. 2, *left*). This repetitive neural activity is transmitted from the motor nerve terminal, producing a brief tetanic contraction which is reflected as an increase in recorded tension. Pretreatment with furosemide reduced the stimulus-bound repetitive activity initiated in the nerve by NaF (fig. 2, *right*), and also reduced the NaF-induced enhancement of muscle contraction. Pretreatment with 1, 10, or 100 μg/kg furosemide reduced the total number of repetitive potentials to 22 ± 15% (n = 5), 17 ± 17% (n = 3), and 13 ± 13% (n = 4) of control, respectively. Pretreatment with 1.0 μg/kg furosemide reduced the duration of repetitive activity to 31 ± 20% of control (n = 5). Furosemide (0.1, 1.0, and 10 μg/kg) reduced the contractile response of muscle to NaF to 13 ± 4% (n = 8), 19 ± 13% (n = 9), and 43 ± 18% (n = 8) of control, respectively.

Pretreatment with furosemide reduced the stimulus-bound repetitive neural activity and the enhancement of muscle contraction initiated by dibutyryl cyclic AMP to about the same degree as it reduced the NaF-induced responses. Specifically, pretreatment with 1.0, 10, and 100 μg/kg furosemide reduced the total number of repetitive potentials to 2 ± 2% (n = 3), 3 ± 3% (n = 3),

and 36 ± 19% (n = 3) of control, respectively; 10 and 100 μg/kg furosemide reduced the duration of repetitive activity to 8 ± 8% (n = 3) and 33 ± 16% (n = 3) of control, respectively, and 0.1, 1.0, and 10 μg/kg furosemide reduced the muscle contraction to dibutyryl cyclic AMP to 2 ± 2% (n = 4), 4 ± 3% (n = 6), and 5 ± 4% (n = 3) of control, respectively.

Theophylline, which enhances cyclic-AMP-mediated effects in many tissues, antagonized furosemide's (1.0 μg/kg) reduction of the nerve and muscle responses to NaF and dibutyryl cyclic AMP. Nerve and muscle responses to NaF and dibutyryl cyclic AMP recorded prior to furosemide did not differ significantly from responses recorded following combined pretreatment with furosemide and theophylline.

Effects of Furosemide at High Doses

Doses of furosemide exceeding 1 mg/kg produced effects on nerve and muscle opposite to those seen following lower doses. These doses (1, 2, and 4 mg/kg) enhanced neural activity, increasing both stimulus-bound repetitive activity and drug-initiated repetitive activity. Figure 3 shows neural activity recorded following 4 mg/kg injection of furosemide; a single nerve stimulus evoked repetitive activity in the motor axon which resulted in increased muscle contraction (not shown). Action potentials in a train of stimulus-bound repetitive activity occurred at a frequency of 300–400 pulses/s, and each train lasted 20–30 ms. The resulting brief tetanic muscle contraction was stronger than the simple twitch of the control period. Furosemide (4 mg/kg) also initiated nerve action potentials that were not bound to be stimulus and appeared immediately after the injection. The drug-initiated repetitive nerve activity lasted 2.5 s, and brought about an asynchronous discharge of action potentials and rapid contractions in the muscle. These contractions increased baseline tension.

The different effects of high and low doses of furosemide were even more apparent with cyclic nucleotide agents. Administration of 1.0–3.2 mg/kg furosemide enhanced the stimulus-bound repetitive nerve activity initiated by NaF, and increased the total number of nerve action potentials to up to 194% of control and the duration of repetitive activity up to 177% of control, (fig. 4). The force of contraction was also increased to 189% of control, (n = 4). Similar doses of furosemide also enhanced stimulus-bound repetitive nerve activity initiated by dibutyryl cyclic AMP. The total number of action potentials and the duration of repetitive activity increased (to 476% and 269% of control, respectively) following injection of 3.2 mg/kg furosemide. The force of muscle contraction also increased to 389% ± 93% of control (n = 3).

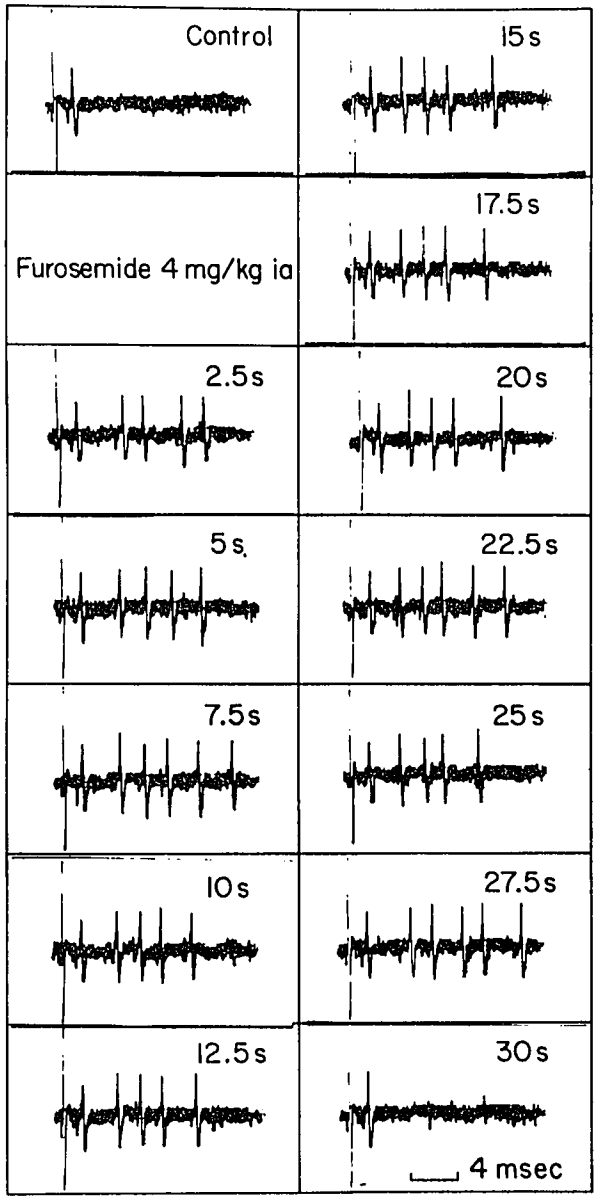

FIG. 3. Stimulus-bound repetitive activity produced in a soleus motor axon by injection of a high dose of furosemide. The first trace on the left shows the response to a single stimulus applied before the drug. Subsequent traces show the responses to similar stimuli applied at the times indicated after the intra-arterial administration of furosemide (4 mg/kg).

The interaction between furosemide and *d*-tubocurarine or succinylcholine were examined as described previously for low doses. Furosemide (1 mg/kg) reversed *d*-tubocurarine-induced neuromuscular blockade (fig. 5*A*). The rate of recovery increased to 678 ± 218% of control (n = 6). Furosemide (2 mg/kg) also reversed the neuromuscular blockade produced by succinylcholine (fig. 5*B*). The rate of recovery increased to 809 ± 254% of control (n = 5).

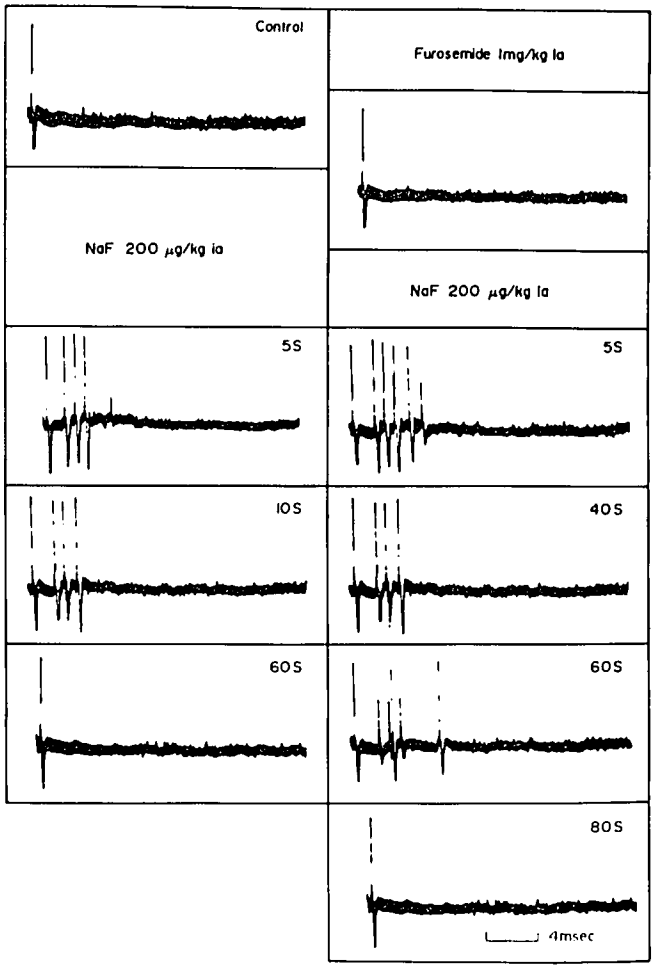

FIG. 4. Effect of furosemide on stimulus-bound repetitive activity produced in a soleus motor axon by NaF. The first trace on the left shows the response to a single stimulus applied before either drug; subsequent traces show the responses to similar stimuli applied at the times indicated after the intra-arterial administration of NaF (200 μg/kg). The first trace on the right shows the response to a single stimulus applied after administration of furosemide (1 mg/kg) and subsequent traces show the responses to similar stimuli administered following combined injections of NaF and furosemide.

Furosemide had no effect on chronically denervated muscle. Forty injections (ranging from 0.1 μg/kg to 8 mg/kg) were administered to five animals. None of these injections initiated contractions in unstimulated muscle or affected the strength of contraction of directly stimulated muscle. Also, pretreatment with furosemide in the doses listed above did not alter the responses of the muscle to injections of acetylcholine (0.5 μg/kg).

Effects of Theophylline and Furosemide on Cyclic AMP Phosphodiesterase Activity

The kinetic characterization of cyclic AMP phosphodiesterase from cat sciatic nerve has been presented elsewhere.[12] However, as observed in other tissues, there were two kinetically distinguishable enzyme activities present in both soluble and particulate fractions from the cat sciatic nerve. Apparent Km values for these two activities were 0.81 μM and 74 μM, in the soluble preparation, and 0.60 μM and 85 μM in the particulate preparation.

High-affinity Cyclic AMP Phosphodiesterase

The ability of theophylline and furosemide to inhibit phosphodiesterase activity was assessed with a Dixon plot.[13] Cyclic AMP hydrolysis was measured in triplicate at three substrate concentrations (0.25, 0.5, and 1.0 μM cyclic AMP), and at five concentrations of each drug. Figure 6 shows a plot from a representative experiment. Both theophylline and furosemide inhibited high-affinity cyclic AMP phosphodiesterase activity in high-speed supernatant and particulate preparations. The apparent Ki values for furosemide were 310 μM and 400 μM for soluble and particulate preparations, respectively, and for theophylline, 440 μM and 250 μM. Each apparent Ki value is the average of two closely agreeing determinations made with separate enzyme preparations. In

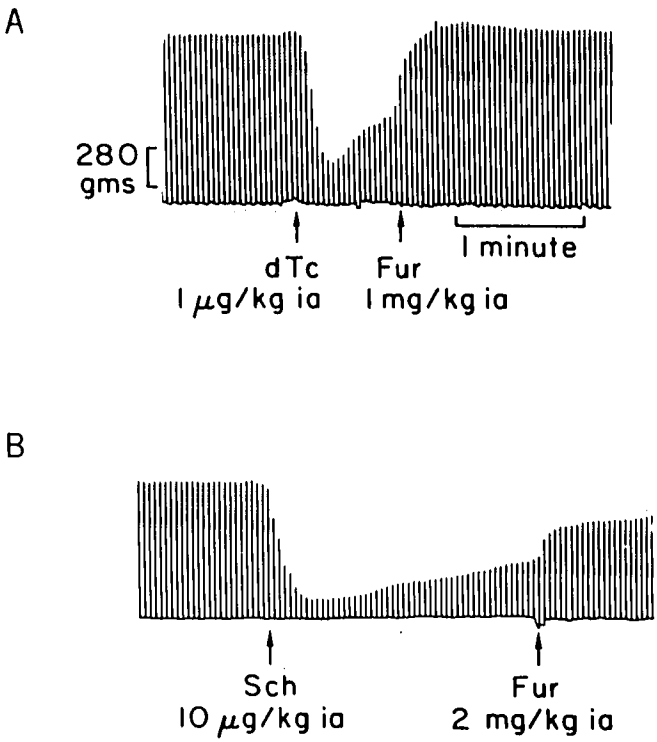

FIG. 5. Record of the effects of furosemide on *d*-tubocurarine and succinylcholine neuromuscular blockade. *A*. Furosemide (1 mg/kg) reversed *d*-tubocurarine blockade; the slope of recovery was 678 ± 218% of control, (n = 6, $P < 0.05$). *B*. Furosemide (2 mg/kg) reversed succinylcholine blockade; the slope of recovery was 809 ± 254% of control, (n = 5, $P < 0.05$).

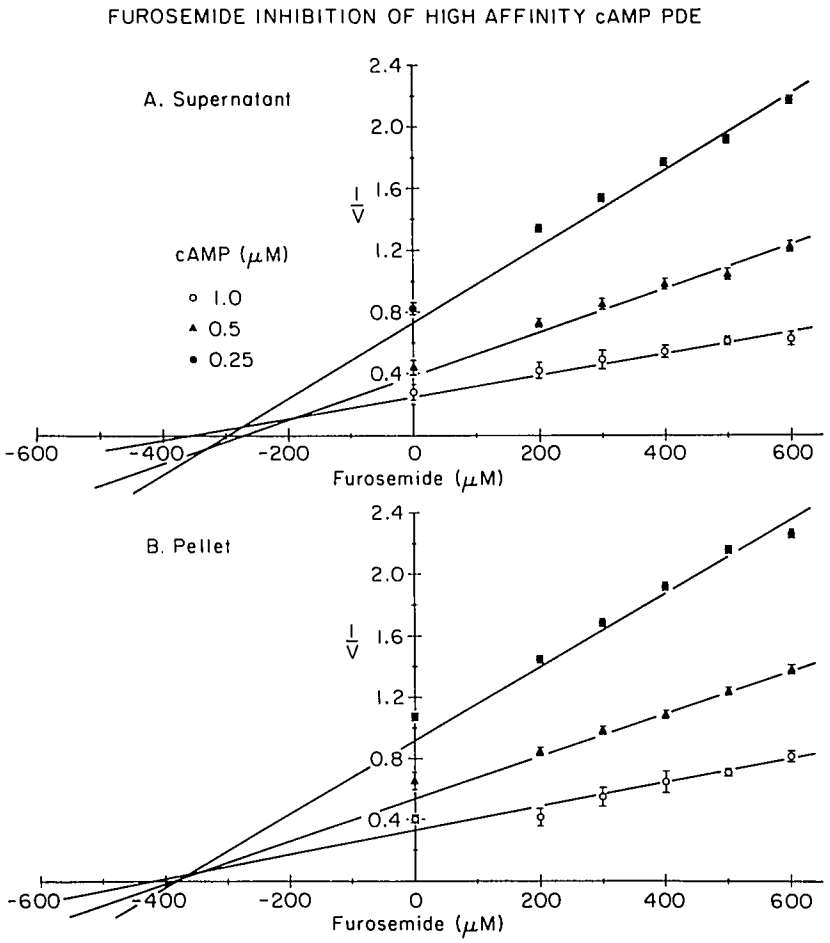

FIG. 6. Dixon plots for determining the apparent Ki of furosemide as an inhibitor of high-affinity cyclic AMP phosphodiesterase; (*A*) particulate fraction, (*B*) soluble fraction. The ordinate is the reciprocal of the velocity of cyclic AMP hydrolysis, expressed as pmol · min^{-1} · mg $protein^{-1}$. The abscissa is furosemide concentration in μM. Three concentrations of cyclic AMP (0.25, 0.5, and 1.0 μM) were used at each furosemide concentration. The point at which the three lines intersect estimates −Ki. The lines represent the best-fitting line as calculated by linear regression using the measurements taken at the five concentrations of furosemide.

all cases, inhibition by theophylline and furosemide was noncompetitive. At very low doses of furosemide (1×10^{-9} and 1×10^{-8} M), no inhibition or activation of either fraction of the high-affinity form of the enzyme was present.

Low-affinity Cyclic AMP Phosphodiesterase

Inhibition of low-affinity phosphodiesterase activity by theophylline and furosemide was assessed at five log concentrations (10^{-7} to 10^{-3} M) of each drug using a single concentration of cyclic AMP (150 μM). A higher, saturating concentration of cyclic AMP was not used because the assay lost sensitivity above 150 μM. Results of these measurements are summarized in table 2. As with high-affinity enzyme activity, theophylline and furosemide produced no significant inhibition of concentrations less than 10^{-5} M. Theophylline caused more inhibition than furosemide at 10^{-4} M, but about the same amount of inhibition at 10^{-3} M.

Discussion

These results indicate that furosemide has a direct effect on neuromuscular transmission. Low doses of furosemide (0.1–10 μg/kg) depress neuromuscular transmission in the *in vivo* cat soleus preparation. They also reduce the concentration of *d*-tubocurarine required to achieve 50% depression of twitch tension in the *in vitro* rat phrenic nerve-diaphragm preparation, and intensify succinylcholine and *d*-tubocurarine blockades in the cat. These results agree with the augmentation of *d*-tubocurarine blockade by clinical doses of furosemide seen in humans.[1] Furosemide had no effect on contractions of denervated muscle evoked by acetylcholine or direct electrical stimulation, thus suggesting that furosemide's effects on innervated muscles are presynaptic.

Low doses of furosemide block the stimulus-bound repetitive neural activity initiated by NaF and dibutyryl cyclic AMP. This effect may be due to furosemide displacing cyclic AMP from specific binding proteins.[14] The neural activity evoked by NaF and dibutyryl cyclic AMP can also be blocked by calcium antagonists such as verapamil and lanthanum.[15] Low doses of furosemide might also act by decreasing calcium influx, which would decrease transmitter output in response to nerve stimulation. This postulated effect might account for

TABLE 2. Drug Inhibition of Low-affinity Cat Sciatic Nerve cAMP Phosphodiesterase

Concentration	Theophylline		Furosemide	
	Specific Activity (pmol · min^{-1} · mg protein^{-1})	Inhibition (%)	Specific Activity (pmol · min^{-1} · mg protein^{-1})	Inhibition (%)
Control (cAMP 150 μM)	552 ± 3*	——	552 ± 3*	——
1×10^{-7} M	526 ± 7	5	579 ± 5	−5
1×10^{-6} M	530 ± 6	4	597 ± 7	−8
1×10^{-5} M	508 ± 6	8	584 ± 4	−5
1×10^{-4} M	436 ± 2	21	559 ± 9	−1
1×10^{-3} M	315 ± 4	43	328 ± 3	40

* Mean ± SE, n = 6 (from two animals each measurement was taken in triplicate).

the decrease in the force of muscle contraction and the potentiation of *d*-tubocurarine and succinylcholine blockades seen in innervated preparations exposed to low doses of furosemide.

In contrast, high doses of furosemide (1–4 mg/kg) enhance and initiate repetitive neural activity, increase the force of muscle contraction, and reverse *d*-tubocurarine and succinylcholine blockades. In *aplysia* neurons, furosemide (10^{-3} M) increases the amplitude of the action potential in a TTX-treated neuron, suggesting that it increases the calcium component of action potentials.[16] This postulated effect would explain the enhancement of transmission seen in cat neuromuscular preparations observed with high doses of furosemide.

The biphasic effects of furosemide were also apparent with cyclic nucleotide agents, since high doses of furosemide potentiated the nerve and muscle responses to NaF and dibutyryl cyclic AMP. These actions of furosemide are similar to those observed with theophylline, a phosphodiesterase inhbitor.[17] Indeed, we found that high doses (1–4 mg/kg) of furosemide inhibit neural cyclic AMP phosphodiesterase, and that the concentration dependence of this inhibition is similar to that of theophylline.

The repetitive neural activity initiated by high doses of furosemide may prolong the afternegativity of the action potential in the motor nerve terminal, thus depolarizing the nerve ending enough to trigger action potentials in the motor axon.[10,18]

In conclusion, furosemide in low doses depresses neuromuscular transmission, depresses the enhancement of transmission produced by NaF and dibutyryl cyclic AMP, and potentiates the neuromuscular blockade produced by *d*-tubocurarine and succinylcholine. In contrast, high doses of furosemide increase the force of muscle contraction, potentiate responses to NaF and dibutyryl cyclic AMP, reverse *d*-tubocurarine blockade, and inhibit phosphodiesterase. These effects of furosemide seem to be exerted presynaptically, and may involve alterations in nerve terminal calcium currents and/or nerve terminal cyclic AMP phosphodiesterase.

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