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The Effect of Nipradilol on the Isoproterenol-Induced Depression of Contractions in the Cat Soleus Muscle

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ABSTRACT—The effect of nipradilol on the isoproterelol-induced depression of contractions of the soleus muscle of the anesthetized cats was studied. Isoproterenol (0.3 μg/kg) injected intravenously decreased the tension and degree of fusion of incomplete tetanic contractions of the soleus muscle of the anesthetized cats. The effect of isoproterenol was blocked by nipradilol (≧3 μg/kg), desnitro-nipradilol (≧10 μg/kg) and propranolol (≧10 μg/kg), but not by nitroglycerin (10–100 μg/kg). Nipradilol (30 μg/kg) and desnitro-nipradilol (300 μg/kg) almost completely antagonized the depressor effects of isoproterenol. These results coupled with evidence that nipradilol does not penetrate the blood-brain barrier indicate that nipradilol exerts an anti-tremor action by blocking peripheral β_2-adrenoceptors.

Keywords: Nipradilol, β-Blocker, Cat soleus muscle, β_2-Adrenoceptor, Isoproterenol

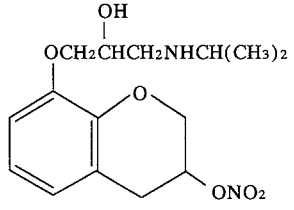
Nipradilol, a peripherally acting β-adrenergic blocking agent, has been used for the treatment of angina pectoris and essential hypertension. Desnitro-nipradilol, which is a metabolite of nipradilol, has weak β-adrenergic blocking activity (1). Figure 1 shows the chemical structures of nipradilol and desnitro-nipradilol. Recently, nipradilol was known to be an effective drug against essential tremor in hypertension patients with essential tremor. β-Adrenergic blocking agents are widely recognized as effective drugs for the treatment of essential tremor, but the precise site of their anti-tremor action is not fully understood. β-Adrenoceptors in the cat soleus muscle are classified as the β_2 type (2, 3), the same as that of slow-contracting human skeletal muscles.

To elucidate the mechanism of the anti-tremor action of nipradilol, we investigated the effect of nipradilol on isoproterenol-induced depression of the tension and degree of fusion of incomplete tetanic contractions of the soleus muscle of the anesthetized cats in this study.

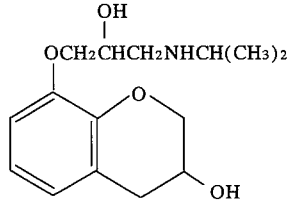
MATERIALS AND METHODS

Care of experimental animals

Adult cats (Keari Co., Ltd., Osaka) of either sex were kept in a clean room that was maintained at 23±2℃, with a relative humidity of 55±10%, 10–20 changes of air per hour and 12 h lighting (7:00–19:00). They were housed in individual stainless steel wire hunger cages ($520^D \times 400^W \times 360^H$ mm) on stainless hunger racks (Keari Co., Ltd.). Solid food (Canet tip fish™; Petline, Tokyo) and tap water in bowls were given to the cats ad libitum. After their normality was confirmed by general obser-



Nipradilol



Desnitro-nipradilol

Fig. 1. Chemical structures of nipradilol and desnitro-nipradilol.

vation, the cats, weighing 2.50–4.74 kg, were used.

Experimental procedure

Adult cats of either sex were anesthetized with a mixture of α-chloralose (80 mg/kg, i.p.) and sodium pentobarbital (6 mg/kg, i.v.). A catheter was inserted into the right carotid artery of each cat for monitoring blood pressure. A cephalic vein was catheterized for the administration of drugs. The animal was laid prone on the operating table and the left hind limb was rigidly clamped in a horizontal position by means of drills through the femur and the tibia and fibula. The skin incision over the insertion of the achilles tendon was extended to the popliteal space where the sciatic nerve was cut. Gastrocnemius muscle and other tissue were retracted to expose the soleus muscle. A small insulated bipolar electrode was placed on the soleus branch of the nerve close to the muscle. The tendon of insertion of the soleus muscle was cut and attached to a FD pickup (TB-611T; Nihon Kohden, Tokyo) connected to a polygraph (RMP-6004, Nihon Kohden). The exposed soleus muscle was covered with liquid paraffin to prevent drying. The rectal temperature and muscle temperature were monitored during the experiment. Hot plate and white light were used to keep these temperatures at 36–38℃. The soleus branch of the nerve was then stimulated at 10-s intervals with a 1-s train of rectangular pulses with pulse duration of 0.1 ms, pulse frequency of 6 or 7 Hz and stimulus voltage of 2 V. This degree of stimulation was determined in the preliminary experiment. At first, physiological saline (as a control) was administered via a chephalic vein at 5 min before the intravenous injection of isoproterenol (0.3 μg/kg). This concentration of isoproterenol (0.3 μg/kg) was determined in the preliminary experiment. Each cumulative dose of drugs (nipradilol, desnitro-nipradilol, nitroglycerin and propranolol) was administered sequentially at 30-min intervals. Isoproterenol was injected intravenously at 5 min after the intravenous injection of these drugs.

Drugs and preparation of test solution

Nipradilol (Lot No. DD) and desnitro-nipradilol (Lot No. 9) were donated by Kowa Company, Ltd. (Tokyo) *dl*-Propranolol hydrochloride (Lot No. ESL7477) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka). Nitroglycerin (millisrol®inj., Lot No. Z60200), a parenteral solution, was purchased from Nippon Kayaku Co., Ltd. (Tokyo). *l*-Isoproterenol hydrochloride (Lot No. 45H5001) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nipradilol (100 mg) was dissolved in 0.1 N HCl (6.7 ml) and then made up to 10 ml with distilled water for injection (Lot No. 6C74N; Otsuka Pharmaceutical Factory, Inc., Tokushima). Desnitro-nipradilol (100 mg) and *dl*-propranolol hydrochloride (100 mg) were dissolved in physiological saline (Lot No. 7B84N, Otsuka Pharmaceutical Factory, Inc.) and then made up to 10 ml with physiological saline, respectively. These drug solutions were diluted with physiological saline to obtain the desired concentrations. Nitroglycerin was used at the original concentration and diluted with saline to obtain the desired concentrations. *l*-Isoproterenol hydrochloride was dissolved in saline to obtain the desired concentration (0.3 μg/kg). Cumulative doses of these drugs were as follows: nipradilol (1, 3, 10, 30 μg/kg), desnitro-nipradilol (10, 30, 100, 300 μg/kg), nitroglycerin (10, 30, 100 μg/kg) and *dl*-propranolol hydrochloride (10, 30, 100 μg/kg) were administered via a cephalic vein with the venous catheter.

Data analyses

Data are expressed as the mean±S.E.M. Statistical analyses were performed by the paired *t*-test. P values less than 0.05 were regarded as statistically significant.

RESULTS

Figures 2 and 3 illustrate representative examples of nipradilol, desnitro-nipradilol, nitroglycerin and propranolol on isoproterenol-induced depression of the tension and degree of fusion of incomplete tetanic contractions of the soleus muscle of the anesthetized cats in this study. Isoproterenol (0.3 μg/kg) decreased the tension and degree of fusion of incomplete tetanic contractions of the soleus muscle. Results of this study are summarized in Table 1. All values of contractions are expressed as the percentages to each value before the administration of isoproterenol as 100%.

As shown in Table 1, isoproterenol (0.3 μg/kg) produced a decrease in the percentage of contractions of the soleus muscle in the saline treated-groups (58.8–62.9%). Nipradilol at the cumulative doses of 1, 3, 10 and 30 μg/kg caused a dose-dependent increase in the percentage of contractions of the soleus muscle to 61.1%, 64.6%, 82.1% and 97.3%, respectively. Nipradilol (30 μg/kg) almost completely antagonized the depressor effects of isoproterenol. Desnitro-nipradilol at the cumulative doses of 10, 30, 100 and 300 μg/kg caused a dose-dependent increase in the percentage of contractions of the soleus muscle to 70.5%, 77.1%, 92.9% and 97.6%, respectively. Desnitro-nipradilol (300 μg/kg) almost completely blocked the depressor effects of isoproterenol. Nitroglycerin at all cumulative dose levels did not affect the depressor effects of isoproterenol. Propranolol at the cumulative doses of 10, 30 and 100 μg/kg caused a dose-dependent increase in the percentage of contractions of the soleus muscle to 65.7%, 75.0% and 90.5%, respectively.

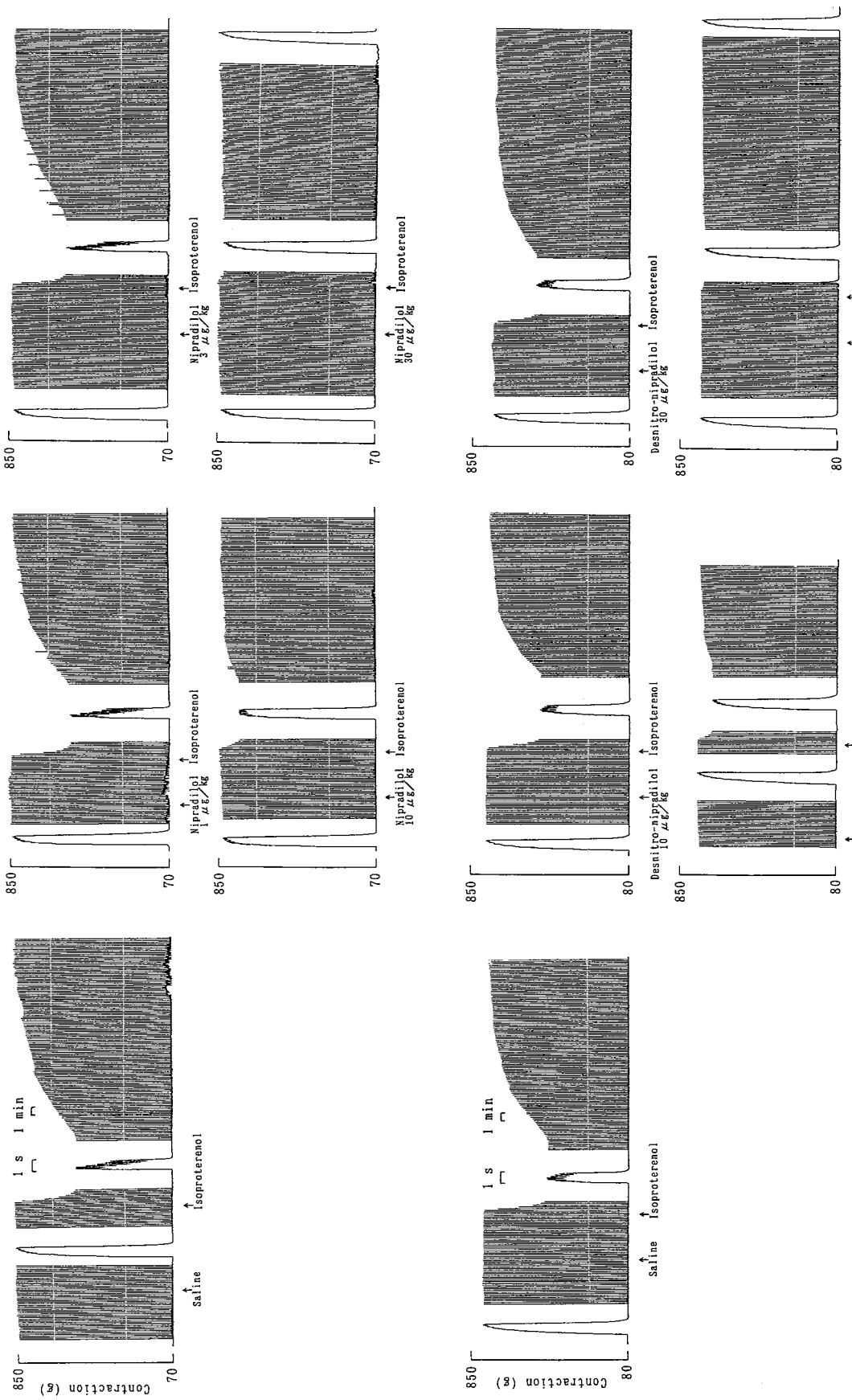


Fig. 2. Effects of nipradilol and desnitro-nipradilol on the isoproterenol-induced depression of contractions in the cat soleus muscle. Contractions of the soleus muscle were evoked by electrical stimulation to the soleus branch of nerve at 10-s intervals with a 1-s train of rectangular pulses with pulse duration of 0.1 ms, pulse frequency of 7 Hz and stimulus voltage of 2 V. Each dose of nipradilol and desnitro-nipradilol was injected intravenously 5 min before the intravenous injection of isoproterenol (0.3 μg/kg).

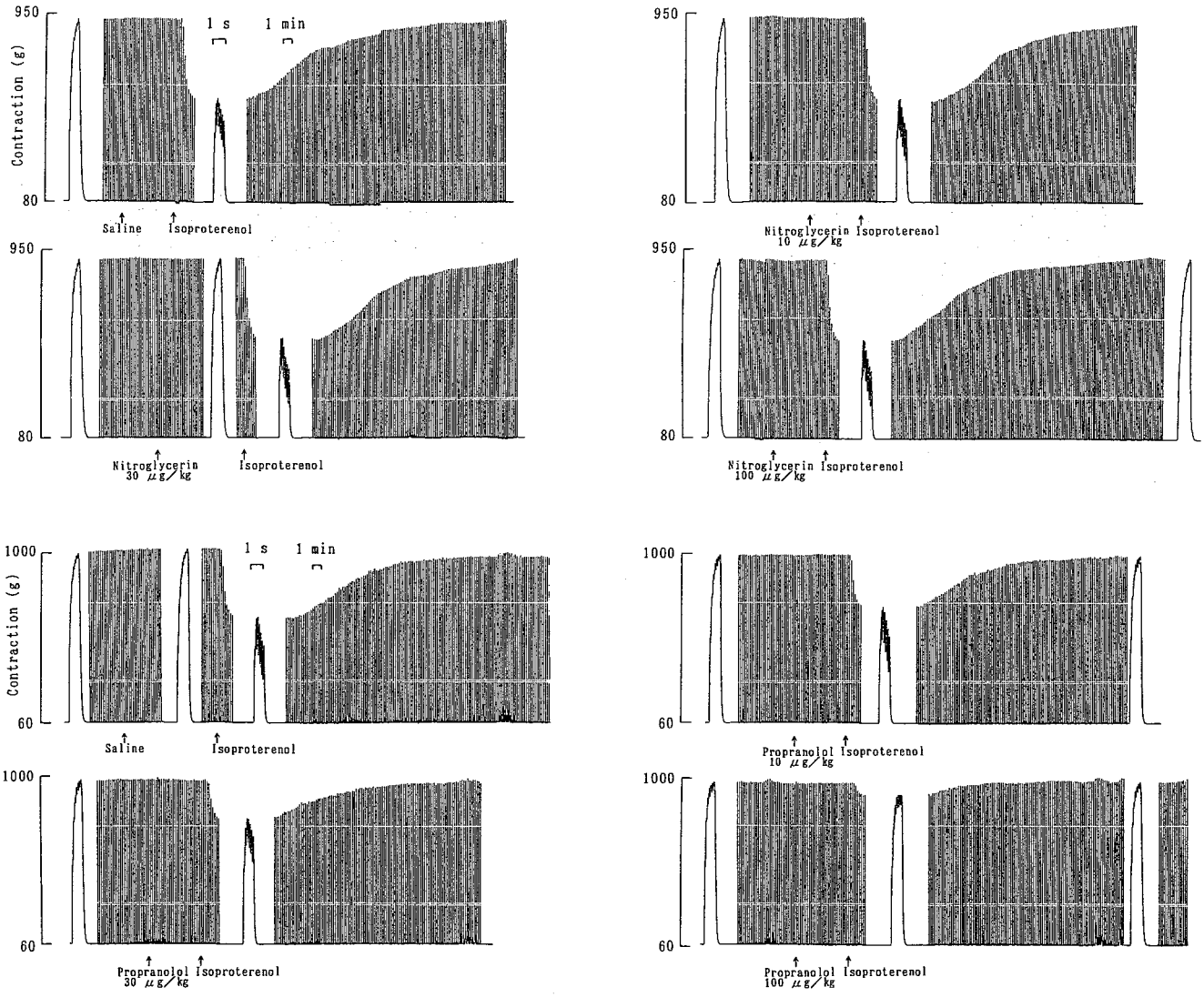


Fig. 3. Effects of nitroglycerin and propranolol on the isoproterenol-induced depression of contractions in the cat soleus muscle. Contractions of the soleus muscle were evoked by electrical stimulation to the soleus branch of nerve at 10-s intervals with a 1-s train of rectangular pulses with pulse duration of 0.1 ms, pulse frequency of 7 Hz and stimulus voltage of 2 V. Each dose of nitroglycerin and propranolol was injected intravenously 5 min before the intravenous injection of isoproterenol (0.3 μg/kg).

DISCUSSION

In the previous studies (4–8), isoproterenol and epinephrine injected intravenously induced depression of the tension and degree of fusion of incomplete tetanic contractions of the soleus muscle of the anesthetized cats. This depression of twitch tension is known to correlate with essential tremor (9). It is known that tremor is potentiated by isoproterenol (10) and epinephrine (9, 11). These effects on the cat soleus muscle are very similar to those on slow-contracting human skeletal muscles (9, 12). β-Adrenoceptors in the cat soleus muscle are classified as of the β_2 type (2, 3) and similar to that of slow-contracting human skeletal muscle. Bowman et al. (2) suggested that the cat soleus muscle may provide a useful test to forecast the possibility of tremor being produced in man.

The frequency of essential tremor usually ranges from 4 to 12 Hz (13, 14) in clinical studies. In this study, the contractions of the cat soleus muscle were evoked by electrical stimulation of the soleus branch of nerve at 10-s intervals with a 1-s train of rectangular pulses with pulse duration of 0.1 ms, pulse frequency of 6 or 7 Hz and stimulus voltage of 2 V. The maximum decrease produced by isoproterenol (0.3 μg/kg) in evoked tension and degree of fusion of incomplete tetanic contractions of the cat soleus muscle occurred with a stimulation frequency of 6

Table 1. Effects of nipradilol, desnitro-nipradilol, nitroglycerin and propranolol on the isoproterenol-induced depression of contractions in the cat soleus muscle

Pretreatment	Cumulative dose (μg/kg, i.v.)	Isoproterenol (μg/kg, i.v.)	Percentage of contractions (Mean±S.E.M.)	n
Saline	0	0.3	59.1±2.1	6
Nipradilol	1	0.3	61.1±2.9	6
	3	0.3	64.6±3.1**	6
	10	0.3	82.1±2.2**	6
	30	0.3	97.3±0.6**	6
Saline	0	0.3	62.9±1.9	6
Desnitro-nipradilol	10	0.3	70.5±1.8**	6
	30	0.3	77.1±2.7**	6
	100	0.3	92.9±2.0**	6
	300	0.3	97.6±0.7**	6
Saline	0	0.3	58.8±1.4	6
Nitroglycerin	10	0.3	58.7±1.7	6
	30	0.3	59.7±2.2	6
	100	0.3	59.8±1.8	6
Saline	0	0.3	60.9±2.5	6
Propranolol	10	0.3	65.7±2.5*	6
	30	0.3	75.0±2.3**	6
	100	0.3	90.5±1.7**	6

Contractions of the soleus muscle were evoked by electrical stimulation to the soleus branch of the nerve at 10-s intervals with a 1-s train of rectangular pulses with pulse duration of 0.1 ms, pulse frequency of 6 or 7 Hz and stimulus voltage of 2 V. Each dose of nipradilol, desnitro-nipradilol, nitroglycerin and propranolol was injected intravenously 5 min before the intravenous injection of isoproterenol. All values of contractions are expressed as the percentages to the each value before the administration of isoproterenol as 100%. *$P<0.05$, **$P<0.01$: Statistical significance of difference from the saline-treated group (paired t-test).

or 7 Hz. The range of these frequencies (6 and 7 Hz) of stimulation of the cat soleus muscle are within the physiological range of frequencies for essential tremor.

In this study, we used the cat soleus muscle as a model of essential tremor. Nipradilol (30 μg/kg) and desnitro-nipradilol (300 μg/kg) almost completely abolished the depressor effects of isoproterenol. Propranolol and desnitro-nipradilol produced almost equal inhibition on the depressor effects of isoproterenol.

On the other hand, nitroglycerin at all cumulative dose levels did not affect depressor effects of isoproterenol. It is well known that nitroglycerin releases nitrite ion rapidly in the body by an enzyme-catalyzed reaction. Nipradilol is biotransformed by an enzyme-catalyzed reaction to yield desnitro-nipradilol and nitrite ion. These results indicate that nitrite ion did not affect the depressor effects of isoproterenol.

It is generally accepted that β-adrenoceptor blockers are often effective in reducing the power of essential tremor, but the pathophysiological basis of essential tremor remains unknown. The mechanism of the anti-tremor action of β-adrenergic blocking agents are as follows (13): 1) β_2-Adrenergic receptor blockade may be critical for anti-essential tremor effects, 2) Intrinsic sympathomimetic activity (ISA) may have a negative impact on anti-tremor effects, 3) The membrane-stabilization effects of β-adrenergic antagonists are of no importance for their therapeutic actions in essential tremor, 4) Anti-tremor agents may be categorized into two major groups: CNS-acting and peripheral nervous-system acting drugs.

In addition, it was reported that the mechanism of the anti-tremor action of β-adrenergic blocking agents might be mediated at a peripheral site (10, 11, 15). Hara et al. (16) proposed that arotinolol, a non-selective β-adrenergic blocking agent, may inhibit β_2-adrenoceptors in the extrafusal muscle fibers of the cat soleus muscle and thereby reduces the amplitude of tremor by changing the incomplete tetanic contractions of the muscle to complete ones.

In humans, the elimination half-lives ($T_{1/2}$) of nipradilol, desnitro-nipradilol and propranolol were 3.7, 7.9 and 2 to 3 h, respectively (14, 17). $T_{1/2}$ values of

nipradilol and desnitro-nipradilol are longer than that of propranolol. β-Adrenoceptor blocking actions of nipradilol, desnitro-nipradilol and propranolol in guinea pig right atrial strips (β_1-adrenoceptor) and trachea (β_2-adrenoceptor) are as follows (1): The potency ratios of nipradilol, desnitro-nipradilol and propranolol on β_1-adrenoceptor blocking actions were 2.63, 0.25 and 1 in guinea pig right atrial strips, respectively. The potency ratios of nipradilol, desnitro-nipradilol and propranolol on β_2-adrenoceptor blocking actions were 2.45, 0.22 and 1 in guinea pig trachea, respectively. Nipradilol shows a non-selective β-adrenoceptor blocking action in isolated guinea pig right atrial strips and trachea, and its action is about 2 to 3 times more potent than that of propranolol. These results correspond with the present study. Recently, nipradilol has been found to be effective against essential tremor in hypertension patients with essential tremor. These findings suggest that administration of nipradilol may be more effective for the treatment of essential tremor than propranolol.

The above findings coupled with evidence that nipradilol does not penetrate the blood-brain barrier indicate that nipradilol exerts an anti-tremor action by blocking peripheral β_2-adrenoceptors.

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