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Studies on a New Beta-adrenoceptor Blocker, 6-(2-Hydroxy-3-isopropylamino)propoxy Benzothiazole Succinate (KF-577)

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Beta-adrenoceptor blocking activity of a newly synthesized benzothiazole derivative, 6-(2-hydroxy-3-isopropylamino)propoxybenzothiazole succinate (KF-577) was investigated. In *in vitro* preparation of the guinea pig trachea and the atrium it was approximately 1/100 and 1/10 as potent as propranolol, respectively, in competitively blocking the beta-receptors: some cardioselectivity was seen. Like propranolol, it is devoid of intrinsic beta-stimulating activity. In urethane-anesthetized rats KF-577 and propranolol both exhibited a pressor effect at lower doses, while at higher doses both caused a sharp fall in blood pressure. The depressor and positive chronotropic effects of isoproterenol were both blocked by either KF-577 or propranolol. Mechanisms of this pressor effect were explored using vagotomized, adrenalectomized, reserpinized rats, and the results were subjected to discussion. KF-577 had a surface anesthetic potency 1/20 that of propranolol (guinea pig cornea) and a conduction anesthetic potency comparable to propranolol (frog sciatic nerve). KF-577 was thus shown to be a beta-adrenoceptor blocker having some selectivity.

Keywords——beta-adrenoceptor blocker; tracheal smooth muscle; atria; cardiovascular effects; local anesthetic effect

Many beta-adrenoceptor blockers appeared since Powell and Slater[2] first described the beta-adrenoceptor blocking effect of dichloroisoproterenol. It is generally accepted that these beta-blockers exert various useful therapeutic effects—antiarrhythmic, antianginal and hypotensive effects—and some workers have suggested that the blockers have some other beneficial effects—remission of essential tremor[3] and of thyrotoxicosis,[4] anxiolytic effect,[5] prophylaxis of migraine,[6] *etc.* These effects relate not always to their beta-blocking activity; some of them relate to so-called "quinidine-like activity", and others to unknown mechanisms not to have been identified as beta-blocking effect.

The effort to search new beta-blockers is not confined in obtaining more potent or more selective beta-blockers but is often focused on finding drugs having beneficial effects frequently associated with beta-blocking effect and having minimal untoward effects.

6-(2-Hydroxy-3-isopropylamino)propoxy benzothiazole succinate (KF-577) was the most potent beta-blocker among the 2-hydroxy-3-alkylaminopropoxy benzothiazoles synthesized as potential beta-blockers.[7] The benzothiazole ring is unique among the hitherto known beta-blockers. This substance was found to have an antiarrhythmic effect com-

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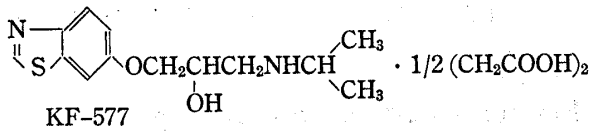

KF-577

Chart 1

parable to propranolol in potency in antagonizing the ouabain-induced arrhythmia in guinea pigs.[8]

This paper outlines the beta-adrenoceptor blocking effect and the general pharmacological properties of KF-577 in comparison with propranolol.

Methods and Materials

1) **Isolated Muscle Preparations**——KF-577 and propranolol were evaluated both for intrinsic activity and ability to prevent the effect of isoproterenol. Isolated tracheal strips and atria from the male guinea pig (350—450 g) were suspended in a 30ml-organ bath containing modified Krebs Ringer (NaCl 120, KCl 4.7, $CaCl_2$ 2.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 14 mM) oxygenated with 95% O_2–5% CO_2 and maintained at 37°. An initial tension of 0.5 g was applied and allowed to equilibrate for 1 hr. Following this, cumulative dose-response curve to isoproterenol was determined isotonically (trachea) or isometrically (atria). The shift of the dose-response curve was determined after 30 min (trachea)- or 10 min (atria)-treatment with KF-577 or propranolol. Beta-adrenoceptor blocking potency was evaluated by determining the pA_2 value, which was the mean of pA_2 values for each preparation.

The negative inotropic and chronotropic effect of KF-577 and propranolol was determined in atria from normal and reserpinized (5 mg/kg, *i.p.*, for one day) guinea pigs.

2) **Local Anesthetic Action**——Frogs were decapitated and the sciatic-tibial peroneal nerve (sciatic trunk) were removed bilaterally and placed in Ringer's solution for frogs (NaCl 6.0, KCl 0.075, $CaCl_2$ 0.1, $NaHCO_3$ 0.1 g/l). The sciatic trunk was then placed in the sucrose gap apparatus and was stimulated at their peripheral ends with square waves of 0.05 msec duration and supramaximal voltage at a frequency of 2 Hz. Spike potentials were photographed every 10 min for 1 hr starting at the time when nerves were continuously perfused with the drugs. The percentage change from control spike amplitude was calculated.

Surface anesthesia produced by the drugs was estimated by instillation of known concentrations of each drugs into the conjunctival sac of the guinea pig lower lid. The lid was held manually closed for 1 min. At regular intervals thereafter, the eye was tested for the presence or the absence of the corneal reflex using a rabbit-whisker. The contralateral eye served as the control.

3) **Cardiovascular Effect *in Vivo***——Male rats of Donryu strain weighing 350—500 g were anesthetized with 1.5 g/kg of urethane, *i.p.*. A cannula inserted into the right carotid artery leading to a strain gauge pressure transducer and the arterial blood pressure and the heart rate were recorded on a polygraph (Nihon-Koden, model RM-150). Drugs dissolved in saline were given into the femoral vein. In a series of experiments the following pretreatments were performed, alone or in combination: bilateral vagotomy, bilateral adrenalectomy, reserpinization (5 mg/kg, *i.p.*, 24 hr before).

4) **Antitremor Action**——Male mice (15—25 g) were used. Tremorine (20 mg/kg, *i.p.*)-induced tremor and hypothermia were assessed according to the scoring methods of Spencer.[9] The effect of the test drugs in either preventing or reversing the effects of tremorine was assessed using two or three dose levels.

5) **Acute Toxicity Test**——Nonfasted male mice of dd-strain weighing 15—25 g were administered subcutaneously with KF-577 and propranolol. The LD_{50} values were determined according to the up-and-down method.

6) **Drugs**——Following drugs were used. Isoproterenol sulfate (Tokyo-Kasei Kogyo Co.), procaine hydrochloride (Sankyo Co.). Propranolol hydrochloride and KF-577 were kindly supplied by Sumitomo-Kagaku Co. and Kyowa-Hakko Kogyo Co., respectively. Doses refer to the salt.

7) **Statistics**——Results are expressed as the mean ±S.E. Student's t test was used to test the significance of differences between means; a statistical inference of significance was made when $p<0.05$.

Results

1) *In Vitro* Guinea Pig Preparations

The isoproterenol dose-response curves for its relaxing effect on the tracheal preparation (Fig. 1) and positive inotropic, chronotropic response in the atrial preparation (Fig. 2) were shifted to the right in a parallel manner after the treatment with KF-577 or propranolol.

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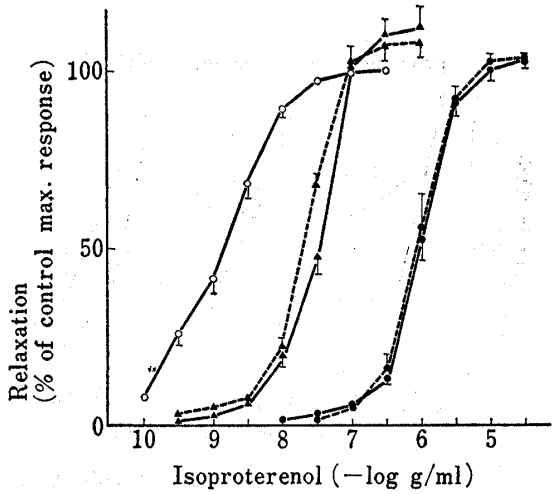

Fig. 1. Shift of the Cumulative Dose-response Curve for the Isoproterenol-induced Relaxation in the Guinea Pig Trachea *in Vitro*

Antagonists were added 30 min before the first dose of isoproterenol. —○—, control (n=19); —▲— and —●—, KF-577, 5×10^{-7} and 5×10^{-5} g/ml (n=5 for each); ---▲--- and ---●---, propranolol, 5×10^{-9} and 5×10^{-7} g/ml (n=5 for each).

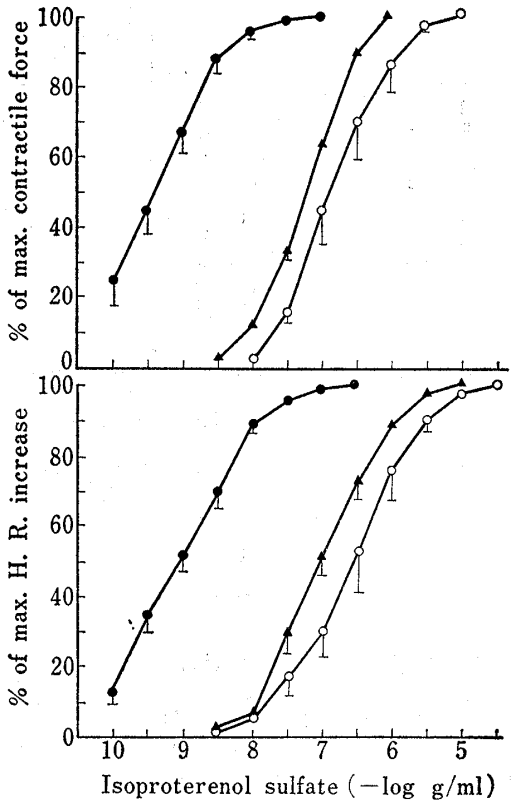

Fig. 2. Dose-response Curve for Inotropic (above) and Chronotropic (below) Responses of Guinea Pig Atria to Isoproterenol

The cumulative dose-response curve was determined 10 min after the addition of antagonists. ●, control; ○, KF-577, 10^{-6} g/ml; ▲, propranolol, 10^{-7} g/ml (n=5).

Table I lists the pA_2 values. These values do not coincide with the shift of dose-response curve shown in Fig. 1 and 2. This is because the pA_2 values were obtained on each preparation after the responses of each preparation were subjected to logit transformation and the control curves in the figures are the mean of all the control dose-response curves. KF-577 was weaker than propranolol as a beta-adrenoceptor antagonist in these *in vitro* preparations. Difference between ED_{50} values of KF-577 in the trachea and in the heart was statistically significant ($p<0.01$), whereas that of propranolol was not ($p>0.05$). KF-577 had therefore some cardioselectivity.

TABLE I. Parameters of KF-577 for the Specific Antagonism to Isoproterenol and for the Cardiac Depression in the Isolated Preparations from Guinea Pigs

	pA_2 for antagonism to isoproterenol			50% effective concn. for negative inotropic effect (M)
	Atria		Trachea	
	Chronotropic effect	Inotropic effect		
KF-577	7.23±0.06	7.31±0.05	6.50±0.19	4.5×10^{-4}
Propranolol	8.28±0.12	8.60±0.33	8.78±0.20	4.5×10^{-5}

KF-577 reduced the force and rate of contraction of the atrium (Fig. 3) at higher concentrations. ED_{50} of KF-577 for the negative inotropic action was 4.5×10^{-4} g/ml, being ten times higher than that of propranolol (Table I).

KF-577 neither exerted any relaxing effect on the tracheal preparation at 10^{-4} g/ml, nor had any demonstrable intrinsic beta-adrenoceptor stimulating action in the atria isolated from normal and reserpinized guinea pigs.

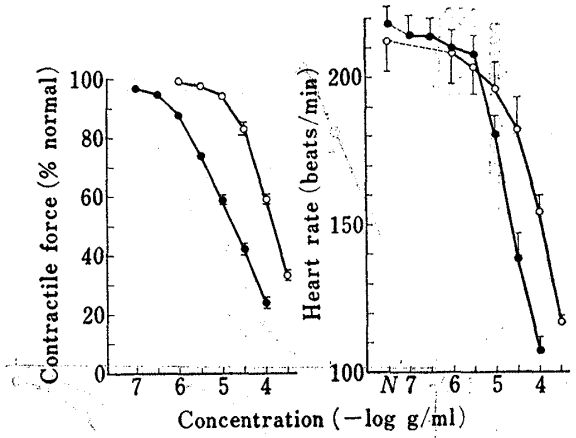

Fig. 3. Decrease in Heart Rate (Right) and Contractile Force (Left) of the Guinea Pig Atria Produced by Cumulative Doses of Propranolol and KF-577

○, KF-577; ●, propranolol.

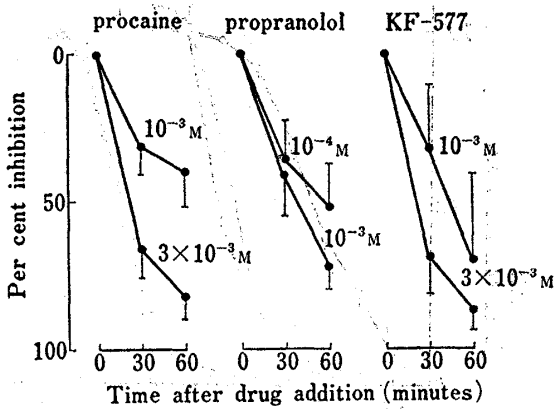

Fig. 4. Time Course of Percent Inhibition in the Action Potenial of the Frog Sciatic Nerve Trunk by KF-577, Propranolol and Procaine

2) Local Anesthetic Activity

The effect of KF-577, propranolol and procaine on the spike potential of the isolated frog sciatic nerve trunk over a period of 60 min was shown in Fig. 4. The results show that KF-577 and propranolol at a concentration of 10^{-3} M produced 73 and 70% reductions in the spike amplitude, respectively, after a 60 min-exposure. At a concentration of 3×10^{-3} M, KF-577 and procaine produced 87 and 82% reductions, respectively. On this basis, KF-577, procaine and propranolol possessed approximately equal potency in reducing the spike potential of frog sciatic trunk.

Table II shows the results of the test for surface anesthesia obtained KF-577, propranolol and procaine. KF-577 was approximately of equal activity to procaine on a w/v concentration basis, while propranolol showed 18 times the surface anesthetic action of KF-577 and procaine.

Table II. Local Anesthetic Effect of KF-577 in the Guinea Pig Cornea

	50% effective concn. (%)	Relative potency
KF-577	1.85	0.93
Propranolol HCl	0.09	18
Procaine HCl	1.73	1.00

3) Cardiovascular Effect

Effect of KF-577 and propranolol on the blood pressure and heart rate under various experimental conditions was studied to determine the possible mechanism of the pressor effect of KF-577.

Fig. 5a and 5b summarize the results obtained. As shown in Fig. 5a, KF-577, 10—1000 μg/kg, and propranolol, 1—100 μg/kg, produced a persistent rise in arterial pressure in normal and vagotomized rats, while the opposite effect was observed when the adrenals were removed. These effects on blood pressure were associated with a marked decrease in the heart rate which was abolished by prior reserpinization (Fig. 5b). At higher doses (≧1000 g/kg), there was sharp fall in blood pressure and further decrease in heart rate. Reserpine (5 mg/kg, *i.p.*, 24 hr before experiment) did not completely abolish the pressor effect of KF-577 and

propranolol, but it flattened the slope of dose-response curves for both blood pressure and heart rate response.

Adrenalectomy in combination with reserpinization caused a fall in basal level of blood pressure and heart rate.

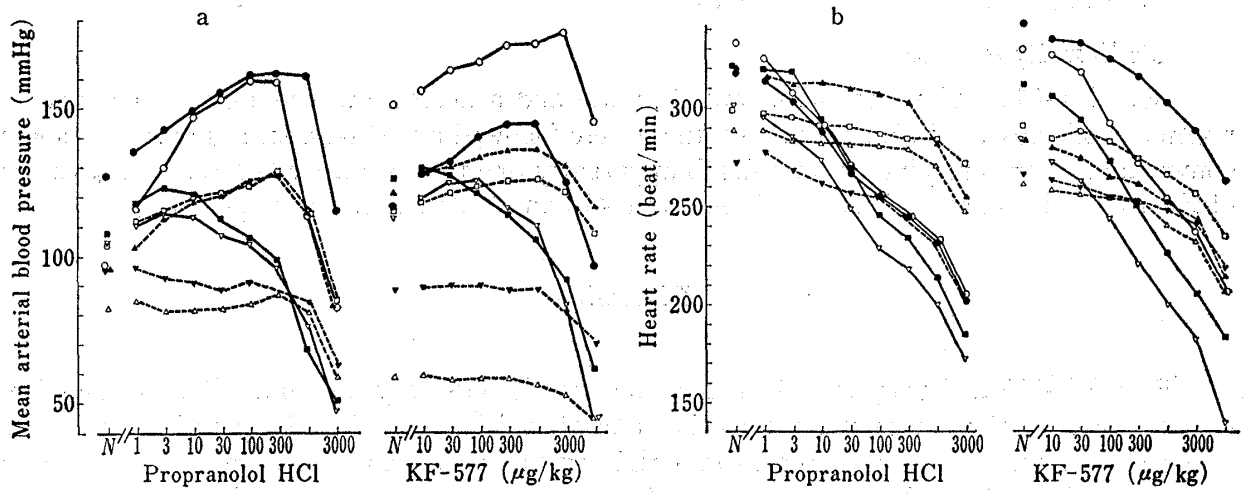

Fig. 5. Influence of Some Treatments on the Pressor Effect (a) and the Negative Chronotropic Effect (b) of KF-577 and Propranolol in Urethane-anesthetized Rats

N: normal level; C: control; A: adrenalectomy; V: vagotomy; R: reserpinization.
Three to five animals were used for each group.
—●—, C —■—, A ---▲---, R ---▼---, R+A —○—, V —▽—, V+A ---□---, V+R ---△---, V+A+R.

The isoproterenol dose-response curve for the depressor effect was shifted to the right in a parallel manner by increasing doses of KF-577, 1, 3 and 10 mg/kg or of propranolol, 0.1, 0.3, 1 and 3 mg/kg (Fig. 6a). Control curves were not parallel to curves after the administration of either beta-blocker, probably because the rise of basal blood pressure level after the smallest doses of the beta-blockers. The parallelism of the shift of the curves for the positive inotropic response was less clear than those for depressor response, because of the gradual decrease in the maximum response with increasing doses of the blockers (Fig. 6b).

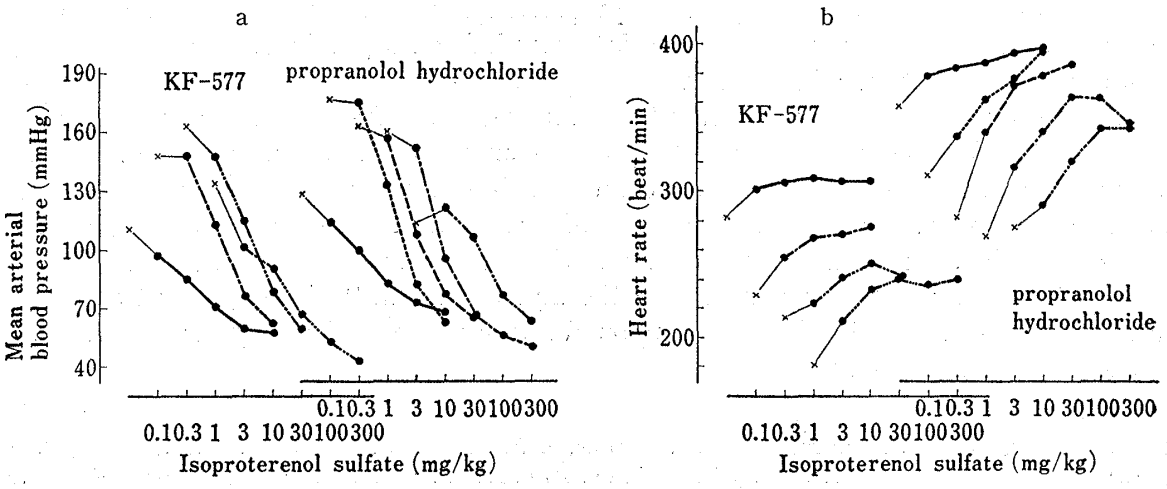

Fig. 6. Influence of KF-577 and Propranolol on the Depressor (a) and the Chronotropic (b) Effect of Isoproterenol in Bilaterally Vagotomized Rats

Results are expressed in percent of maximum change ± S.E.M. $n=3$ to 5.
——— 0 ------- 0.1 ———— 0.3 —·—·— 1 ·—·—·— 3 ———— 10 mg/kg.

4. Antitremor Activity

All drugs were given *i.p.* 1 hr before or after tremorine, 20 mg/kg. Tremor, parasympathetic response (lacrimation, salivation and diarrhea) and hypothermia induced by tremorine were prevented and/or reversed by 10 mg/kg of atropine. Pretreatment with KF-577 (50 and 70 mg/kg) or with propranolol (10 mg/kg) reduced the extent of tremor and hypothermia but both drugs were unable to reverse established tremor and hypothermia.

5. Acute Toxicity in Mice

Both KF-577 and propranolol caused ataxia and a decrease in spontaneous activity at minimal toxic dose. With larger doses, these were followed by depression, tail reaction, clonic convulsion, labored respiration and death apparently due to respiratory failure. LD_{50} values of KF-577 and propranolol hydrochloride were 470 and 300 mg/kg, *s.c.*, respectively.

Discussion

Both KF-577 and propranolol fulfilled the criteria necessary for competitive antagonism of beta-adrenoceptors on guinea pig trachea and atria. They produced a parallel shift of the dose-response curves of isoproterenol to the right and there was no depression of the maximum response to isoproterenol after high doses of these antagonists. A comparison of pA_2 values of KF-577 and propranolol against isoproterenol indicates that KF-577 was less potent than propranolol as a beta-blocker. When the ED_{50} values of KF-577 in tracheal and atrial preparations are compared, not remarkable but statistically significant cardiac selectivity was recognized.

KF-577, like propranolol, had no demonstrable intrinsic beta-adrenoceptor stimulating activity when assessed on the atria isolated from the reserpine-pretreated guinea pigs, but at higher doses it depressed the resting atrial rate and contractile force. These effects became most pronounced after a total cumulative dose of 0.1—0.3 mg/kg. This cardiac depressant effect of KF-577 approximately paralleled its beta-adrenoceptor blocking activity as compared with propranolol.

In addition to its blocking activity on cardiac and bronchial beta-receptors, propranolol is generally believed to possess also membrane stabilizing, so-called quinidine-like actions.[10] These effects of propranolol have been implicated in the antiarrhythmic action of this agent.[11] When applied to the guinea pig cornea (surface anesthesia), the local anesthetic potency of KF-577 was approximately 1/20 that of propranolol, and comparable to that of propranolol in depressing the spike potential of frog sciatic trunk *in vitro* (conduction anesthesia). These results are of interest in relation to the fact that KF-577 was shown to possess a potency comparable to that of propranolol in antagonizing the ouabain-induced arrhythmia in guinea pig *in vivo*.[8] Ritchie and Greengard[12] proposed in their discussion on local anesthetic activity that the effectiveness of a local anesthetic is determined by two factors: 1) the penetration from the site of application to the site of action and 2) the actual anesthetic action at the site of action. In addition, the cation is the active form for local anesthetics[13] but the uncharged form is needed for penetrating the myelin sheath.[14] The apparent greater anesthetic potency of KF-577 when tested upon the frog nerve might suggests a greater ability to penetrate the myelin-sheath.

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Propranolol has been known to have a significant pressor effect in rats[15,16] and dogs.[17] While "unmasking of effects on vascular alpha-receptors" and "catecholamine release from the adrenal medulla" by beta-receptor blocker have been proposed as the mechanisms underlying the pressor effect,[16] Regoli[15] pointed out the dissociation of pressor and beta-blocking effects of some beta-blockers. KF-577 also showed a pressor effect similar to propranolol in anesthetized rats (Fig. 5). We explored whether the pressor effects of these drugs were caused by the same mechanism, using cumulative dose-response curve in normal, vagotomized, reserpinized and adrenalectomized rats, but no indication has been obtained that both drugs have different mechanisms each other.

The hypothesis of "unmasking of alpha-adrenoceptor stimulation" is consistent with the results of the present investigations, and it is very likely that the circulating catecholamines released from adrenals contributes to this effect, but beta-blocker-induced accelerated release of catecholamines from the adrenal could not be substantiated.

The bradycardia induced by beta-blockers is likely to be caused by the blockade of cardiac sympathetic drive and this bradycardia is considered to be a main cause of depressor effect of lower doses in adrenalectomized rats, in which circulating catecholamines are assumed to be greatly reduced.

Propranolol has been shown to have beneficial effect on essential tremor.[3] In animal experiments, it has been suggested that the antitremor action of beta-blockers may not be due to the blockade of peripheral adrenergic beta-receptors, but to other pharmacological action.[18] In the present study, KF-577 and propranolol prevented the tremorine-induced tremor and hypothermia to some extent, while they did not reverse established tremor nor hypothermia.

In summary, it was shown in the present studies that KF-577, which was previously shown to have antiarrhythmic effect comparable to propranolol, had beta-blocking activity appreciably weaker than that of propranolol but had no demonstrable beta-stimulating activity. It is interesting that KF-577 had a conduction anesthetic activity comparable to propranolol but its surface anesthetic activity was appreciably weaker than that of propranolol. The beta-blocking activity showed some $beta_1$-selectivity. The benzothiazole ring could be one of useful substituents which enable us to obtain beta-blockers having selectivity to $beta_1$-receptors.

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