Genesis of Bifid T Wave in Rats

Min Do HUH, Hisashi HIROSE, Shigeru SUGANO, and Hiroshi SAWAZAKI

Department of Animal Environmental Physiology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

(Received 15 July 1985/Accepted 13 November 1985)

ABSTRACT. When the body temperature of a male rat was lowered under anesthesia, it was recognized that the electrocardiographic T wave was consistently altered to a characteristic bifid T feature at rectal temperatures roughly between 30° and 20°C. The prolongation of QT interval was marked with the decrease of rectal temperature. This bifid T wave was restored to its original monofid one by artificially raising the rectal temperature above 30°C or further lowering below 20°C. The bifid T wave returned to normal by propranolol (4 mg/kg, i.p. or i.v.), or by the infusion of procaine solution (2%, 0.2 ml) into spinal subarachnoid space at the level of cervical region (C₁-C₂). On the other hand, from the rat pretreated with reserpine (5 mg/kg, i.p.), no bifid T wave was observed even under hypothermia. The prolongation of QT interval in relation to the body temperature in the reserpine-pretreated rat was less marked than that in the nonpretreated rat. The normal T waves in the normothermic rat as well as in the reserpine-treated normothermic and hypothermic rats were transiently altered to bifid ones by the intravenous administration of isoproterenol (3 µg/kg). These results led the authors to conclude that the hypothermia causes an increase in sympathetic nervous activity in rats and that the bifid T wave is mainly due to the augmented activation of cardiac adrenergic β receptor.—**KEY WORDS:** autonomic nervous system, bifid T wave, hypothermic rat.

Jpn. J. Vet. Sci. 48(1): 137–144, 1986

Some reports indicate that the electrocardiographic bifid or notched T wave is attributable to the unsynchronized repolarization processes occurring in certain portions of the heart ventricles [9, 12]. The abnormal ST-T wave in the dog was also attributed to the imbalances between right and left sympathetic nervous activity [10]. Recently we have observed the appearance of bifid T wave in a hypothermic rat. The effect of immersion hypothermia on the cardiovascular system was extensively studied for possible use in cardiac surgery. The ice-water immersion method was also frequently used for the induction of gastric ulcers in the rat [15, 16]. Nevertheless, the appearance of bifid T wave in hypothermic rats has never been described.

Studies on the experimentally induced gastric ulcer in the rat indicated that the genetic susceptibility to the ulcer is closely related to the autonomic nervous activity [16]. More-

over, the hypothermic condition has been known to increase the efferent sympathetic nervous activity, possibly due to the augmented efferent discharge of nervous impulses from the cold receptors in the skin or other mucous membrane structures [1, 2].

The present study was performed to determine whether or not the autonomic nervous system plays a role in the genesis of the bifid T wave. Rats showing normal or bifid T wave in electrocardiography were treated with drugs to block or stimulate their autonomic nervous systems.

MATERIALS AND METHODS

Animal preparations and recordings: Forty male Wistar rats between 3 and 20 weeks of age were used (>8 rats/experiment) as described below. All the experiments were done with the rats anesthetized with intraperi-

toneal urethan (1.0–1.5 g/kg) except in the specified surgical procedures in which pentobarbital sodium (40 mg/kg, i.p.) was used.

Immediately after the anesthetic induction, rats were tied in the supine position, or occasionally in the sternal position, to a wooden block board and placed with the subcutaneous enamel-coated copper electrodes for ECG recording (lead II). A thermocouple (TF-DN, Terumo Co.) was gently inserted into the rectum. All electrocardiographic signals were amplified (time constant: 1.5 sec) with a bioamplifier (1205C, Nihon Denki San-ei Co.) and recorded on a 4 channel pen oscillograph (RECTI-HORIZ-8K, Nihon Denki San-ei Co.) or a 2 channel recticorder (RJG-3002, Nihon Kohden Kogyo Co.). Simultaneously, the amplified analog signal segments were fed into a signal processor (7T08, Nihon Denki San-ei Co.), and their 50 comparable signal segments were averaged for the accurate analyses of ECGs. Only the signalized ECG waves were represented in the present paper.

Blocking procedures of the autonomic nervous system: Rats were immersed in a cold water bath and removed as soon as the rectal temperature reached 27°C; the bifid T waves were always observed, starting at around 30°C of rectal temperature. Phentolamine (10 mg/kg, i.p.), propranolol (4 mg/kg, i.p. or i.v.), or atropine (1 mg/kg, i.p. or i.v.) was administered at that time. The ECGs were obtained before the administration, the first successive 90 sec, and 2, 3, 5, 7 and 10 min after the administration of each drug.

For the intraspinal injection of procaine, rats were placed at the sternal position after the body temperatures were lowered by the procedure described above. The head was kept high through tilting the animal board at an angle of about 10°C to the horizontal. The 1st and 2nd cervical vertebrae were posteriorly exposed and then, 0.2 ml of 2% procaine solution was slowly injected into intervertebral space by a tuberculin syringe. The sam-

pling method of ECG was the same as described in autonomic blockades.

Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.), the bilateral adrenal glands were exposed by smoothly pulling aside the intestinal loop. The cortical capsules were carefully incised by a scalpel and the glands were squeezed together by the tips of forceps [17]. Reserpine (5 mg/kg) was administered intraperitoneally 3 days after the bilateral enucleation of adrenal glands. As the body temperature reached to between 30° and 26°C about 24 hours after the reserpine administration, the rat was warmed to the normal range of the rectal temperature. Subsequently, the rectal temperature was again lowered to about 27°C by cooling. The recording of ECG was continuously performed at the different rectal temperatures during the cooling.

Stimulating procedures of sympathetic nervous system: Some normothermic rats with the normal T wave received isoproterenol (3 µg/kg) or norepinephrine (10 µg/kg). The reserpine-treated rats received only isoproterenol under either normothermic or hypothermic conditions. These drugs were administered in 0.5 ml via a polyethylene tube cannulated into the jugular or femoral vein. The injection took place in 20 seconds, and the ECG was taken before and during the 10 min period after the injection.

RESULTS

Hypothermia-induced bifid T wave: Fig. 1A shows the electrocardiogram recorded from the normothermic rat through the limb leads I, II, and III. The T wave is characterized by the ascending limb having a steeper angle than the descending limb with the absence of a distinct isoelectric ST segment. When the rectal temperature was lowered to around 30°C, the initial change of T wave occurred in such a way that the end of T wave was extended over the beginning of the next P

Fig. 1. ECGs in limb leads I, II and III recorded from rats with normal and lowered body temperatures. A: Under normothermia. The relatively prominent bifid T wave can be seen in leads II and III under hypothermia (B). B.T.: body temperature.

wave. Simultaneously, the cardiac rate was changed. With further decrease of the rectal temperature to about 27°C, the change of the T wave became more prominent and notched at descending limb of T wave, resulting in a bifid wave. The prominent bifid T wave was always observed when the ECG was obtained through the limb lead II or III (Fig. 1B). The voltage of all deflections of the electrocardiogram was increased as the rectal temperature declined. The bifid T wave thus induced was transformed back to the normal wave either by the artificial rewarming to the normal range of rectal temperature or by lowering it

Fig. 2. The effect of propranolol on hypothermia-induced bifid T wave. The normal deflection of T wave (A) was altered to bifid T wave (B). The administration of propranolol (4 mg/kg, i.p.) resulted in the restoration to normal (C).

to below 20°C.

Blocking effect of autonomic nervous system: The upper 2 traces of Fig. 2 are the ECGs obtained under normothermia (A) and hypothermia (B). With the administration of propranolol, the hypothermia-induced bifid T wave gradually returned to the normal pattern together with a lowered heart rate (Fig. 2C; obtained about 1 min after the administration of propranolol). Little effect on the bifid T

Fig. 3. The effect of intraspinal procaine on hypothermia-induced bifid T wave. A: Before the intraspinal injection of procaine. The application of procaine solution (2%, 0.2 ml) to the level of intervertebral space between C₁ and C₂, led bifid T wave (B) to the normal T wave (C).

Fig. 4. ECGs recorded from reserpine (40 mg/kg, i.p.)-pretreated rat under normothermic and hypothermic conditions. A: Under normothermia, B: Under hypothermia. No recognizable change in T wave is seen in the low body temperature.

wave was observed by the administration of phentolamine or atropine (not presented).

The hypothermia-induced bifid T wave completely returned to normal about 2 min after the intraspinal injection of procaine solution (Fig. 3). The normalization of bifid T wave was accompanied by the removal of electromyographic noises with further decrease in the cardiac rate.

Fig. 4A shows the ECG obtained after reserpine treatment. The change into bifid T wave was not observed during the low body temperature. The ECG recorded at a rectal temperature of 27.5°C is shown in Fig. 4B. The upper trace of Fig. 5 shows the relationship between the rectal temperatures and the

Fig. 5. Relations between rectal temperature and RR interval (upper), and between QT and RR interval (lower) in intact and reserpinized rats. Circular and triangular symbols represent two intact and reserpinized rats, respectively. The portions under distinguishable bifid T wave were indicated as the filled circles.

RR intervals. The lengthening of RR interval was prominent and linearly correlated with the decrease of rectal temperature as compared with that of the non-pretreated rat. The non-pretreated rat showed a transient shortening of RR interval near 35°–33°C and the subsequent gradual lengthening with further decrease in the rectal temperature. The interval, however, remained below the control value until the rectal temperature reached near 28°C. The relationship between RR and QT interval during the cooling is also shown in the lower trace of Fig. 5. No marked lengthening in QT interval was observed from the reserpine-pretreated rat under hypothermia. There was no further lengthening in QT

Fig. 6. The effect of intravenous isoproterenol (3 μ g/kg) on normal T wave of normothermic rat. Normal T wave (A) was altered to bifid T wave (B) following the intravenous injection of isoproterenol.

interval over 110 msec despite the continuous decrease in rectal temperature. On the contrary, the QT interval in the non-pretreated rat continuously lengthened with the decrease of rectal temperature and the appearance of bifid T wave.

Stimulating effect of sympathetic nervous system: The administrations of adrenergic agonists were performed on the normothermic non-treated and reserpine-treated rats with normal T waves. The lower trace of Fig. 6 shows an example of the ECG with bifid T wave (B) signalized 2 min after the intravenous injection of isoproterenol to the normothermic rat with normal T wave (A). The heart rate was markedly increased as compared with that of the control. However, no appreciable response of T wave to the intravenous administration of norepinephrine was observed.

Fig. 7. The effect of intravenous isoproterenol ($3 \mu\text{g/kg}$) on normal T wave of reserpine-treated rat. After the administration of isoproterenol, the T waves were changed to bifid T waves in the both normal (A) and low rectal temperatures (B).

On the other hand, by the intravenous injection of isoproterenol to the reserpine-treated rats, the T waves were also changed to bifid T waves (45 sec after) with increased heart rates, regardless of their body temperatures (Fig. 7).

DISCUSSION

An inversion or flattening of T wave and notched T wave as well as bifid T wave means the ventricular electric deviation even if the primary causes in these abnormalities may be

different. Hitherto, some studies have been performed concerning the genesis of the alteration of the T wave. For example, it has been suggested that the bifid T wave occasionally observed in healthy young rabbits might be due to delayed repolarization in certain portions of the ventricle [2], and that the notched T wave in a normal human might be associated with a marked delay or inhomogeneity in the repolarization process of some part, particularly of the right ventricle [9]. It has been considered that the autonomic nervous tone regulating the cardiac function might

play a primary role in such T wave changes [9, 12].

In the present study, the decrease in the body temperature (30°–20°C) always resulted in the characteristic bifid change of T wave in the rat, and the restoration of bifid T wave towards a normal one was observed under a body temperature of below 20°C. Generally, cold stimulation has been known to result in the increase of sympathetic nervous activity [3]. Walsh [18] found that the sympathetic tone in the rat was very high and that the net autonomic tone was highly positive at a low body temperature of 28° to 24°C. Moreover, Suda *et al.* [14] reported that a progressive suppression in the electrical activity of the central nervous system was observed below 25°C although hyperresponsiveness was produced between the body temperature of 35° to 25°C. These facts suggest that the bifid change of T wave observed in the present study may result from the functional deviation of autonomic nervous system, possibly due to hypothermia.

The hypothermia-induced bifid T wave disappeared with the intraspinally injected procaine. The simultaneous abolishment of electromyogram and the cardiac slowing mean the conduction failure of nervous impulses in both sympathetic and somatic system. Moreover, the administration of propranolol, an adrenergic β blocking agent, also resulted in the normalization of the bifid T wave, while there were no influences of phentolamine (an adrenergic α blockade) or atropine (a cholinergic blockade) on the bifid T wave. The fact implies a predominant intervention of cardiac β receptor to the change of T wave. In the catecholamine-depleted state by reserpine pretreatment, no bifid T wave was observed under hypothermic condition. Accordingly, it was assumed that the rat's bifid T wave under hypothermia was derived from the increased sympathetic nervous activity, so that it could be abolished by an adrenergic β antagonist or by a procedure disturbing nerv-

ous conduction.

In the present study, the QT interval progressively lengthened with the subsequent appearance of bifid T wave during the cooling of rat (see Fig. 5). Willard *et al.* [19] reported that the corrected QT interval in the rat was decreased with cooling and increased with warming. Other investigators, however, reported about the lengthening of QT interval with the cooling of body temperature in the rat, cat and dog [4–8, 11]. It was explained by Hegnauer *et al.* [6] that the lengthening of QT interval may be the effect of the lowered cardiac temperature on the metabolic processes of the heart. However, we did not observe the severe lengthening of QT interval as well as the bifid T wave in the reserpine-treated rat when the body temperature was lowered. Moreover, the intravenous isoproterenol in the reserpine-pretreated hypothermic rat resulted in the marked lengthening of QT interval with the appearance of the bifid T wave. On the other hand, Noda *et al.* [13] and Abildskov [1] reported that isoproterenol or other catecholamines produced a prolongation of QT interval. Accordingly, the marked QT prolongation accompanied by the bifid T wave in the hypothermic rat would be the effect of the increased endogenous catecholamines as the transmitter of sympathetic nervous system rather than that of the lowered cardiac temperature.

REFERENCES

1. Abildskov, J. A. 1976. Adrenergic effects on the QT interval of the electrocardiogram. *Am. Heart J.* 92: 210–216.
2. Anderson, B. E. 1977. Temperature regulation and environmental physiology. pp. 686–695. *In: Dukes' physiology of domestic animals*. 9th ed. (Swenson, M. J. ed.). Cornell University Press, London.
3. Appenzeller, O. 1982. The autonomic nervous system. 3rd ed., Elsevier Biomedical Press, Amsterdam, New York, Oxford.
4. Chrismon, J. M. 1944. Effect of hypothermia on the heart rate, the arterial pressure and the electro-

- cardiogram of the rat. *Arch. Int. Med.* 74: 235-243.
5. Dahlen, R. W. 1964. Some effects of hypothermia on the cardiovascular system and ECG of cats. *Proc. Soc. Exp. Biol. Med.* 115: 1-5.
  6. Hegnauer, A. H., Shriber, W. J., and Haterius, H. O. 1950. Cardiovascular response of the dog to immersion hypothermia. *Am. J. Physiol.* 161: 455-465.
  7. Hirose, H., Suzuki, A., and Sawazaki, H. 1975. Electrocardiographic studies on cardiac arrest in the dog. *Jpn. J. Vet. Sci.* 37: 65-73.
  8. Hook, W. E., and Stormont, R. T. 1941. Effect of lowered body temperature on heart rate, blood pressure, and electrocardiogram. *Am. J. Physiol.* 133: 334-335.
  9. Ishikawa, K., and Ohnuma, H. 1979. The significance of a notch on the T wave. *Jpn. Circ. J.* 42: 539-546.
  10. Kato, K., Iinuma, H., and Takahashi, N. 1978. ST depression induced by sympathetic cardiac nerve stimulation. *Shinzo*. 10: 783-790 (in Japanese).
  11. Nardone, R. M., Wilber, C. G., and Musacchia, X. J. 1955. Electrocardiogram of the opossum during exposure to cold. *Am. J. Physiol.* 181: 352-356.
  12. Nishimura, M., Watanabe, Y., and Toda, H. 1984. The genesis of bifid T waves: experimental demonstration in isolated perfused rabbit heart. *Inter. J. Cardiol.* 6: 1-14.
  13. Noda, M., Kawano, O., Uchida, O., Sawabe, T., Saito, G., and Fukawa, K. 1970. Myocarditis induced by sympathomimetic amines. *Jpn. Circ. J.* 34: 7-12.
  14. Suda, I., Koizumi, K., and Brooks C. M. 1957. Analysis of effects of hypothermia on central nervous system responses. *Am. J. Physiol.* 189: 373-380.
  15. Takagi, K., and Okabe, S. 1968. The effects of drugs on the production and recovery processes of the stress ulcer. *Jpn. J. Pharmac.* 18: 9-18.
  16. Takagi, K., Okabe, S., Yano, S., Kawashima, K., and Saziki, R. 1969. The effect of propranolol on the stress and shay ulceration in rats. *Jpn. J. Pharmac.* 19: 327-329.
  17. Takenaka, F., Ishihara, T. and Maruyama, Y. 1974. Effect of 2-acetyl-7-(2-hydroxy-3-isopropylamino)propoxy benzofuran hydrochloride, new beta-receptor blocking agent on cardiovascular system. *Folia pharmacol. japon.* 70: 385-397 (in Japanese).
  18. Walsh, R. R. 1969. Heart rate and its neural regulation with rising body temperature in anesthetized rats. *Am. J. Physiol.* 217: 1139-1143.
  19. Willard, P. W., and Horvath, S. M. 1959. Electrocardiographic studies on rats before and after cardiac arrest induced by hypothermia and asphyxia. *Am. J. Physiol.* 196: 711-714.

要 約

ラットにおける2峰性T波の成因について：許 敏道・広瀬 昶・菅野 茂・澤崎 坦（東京大学農学部家畜環境生理学教室）——麻酔下で雄ラットの体温を 30°C～20°C の間に下降させると、心電図 QT 間隔の延長に伴ってT波の2峰化が観察されたが、体温が 30°C 以上あるいは 20°C 以下になると対照波形に回復した。2峰性T波の成因に自律神経の活動状態が関連しているかどうかを調べる目的で、自律神経作用薬を用いて検討した結果、プロプラノロール（4 mg/kg）の腹腔内あるいは静脈内投与、また上頸部脊髓腔内へのプロカイン溶液（2 %, 0.2 ml）注入によって2峰性T波の正常化がみられたが、レセルピン（5 mg/kg, i. p.）前処置によっては、体温を下降させてもT波の2峰化は認められず、QT の延長も非処置ラットに比べ軽度であった。一方、正常T波を示す正常体温およびレセルピン前処置ラットに対してイソプロテレノール（3 μg/kg）を静脈内に投与すると、T波の2峰性化が認められた。以上の成績から、低体温において出現するラットの2峰性T波の成因として、とくにβ受容体を介する交感神経系の緊張度が深く関与していると推測された。