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Effects of Sodium Fluoride on Frog Heart Motility

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Summary

Effects of sodium fluoride (NaF) on the bullfrog heart were investigated. NaF (10 mM) inhibited the autonomic heart contraction, reducing the heart rate. This inhibitory action was recovered by perfusion with 2-Ca^{+} Ringer's solution. The twitch tension of auricular muscle was reduced by 2 mM NaF as well as 1 mM sodium oxalate and low-Ca^{+} Ringer, but the latter two were less effective than the former. However, NaF (5 mM) induced a rise in resting tension similar to that in the case of DNP application. ATP, adrenaline, and theophylline all reversed the inhibitory action of NaF, while both DNP and iodo acetic acid promoted it. NaF (5 mM) decreased the tension of Na^{+}-deficient contracture. The results obtained suggest that the reduction in the heart motility by NaF might be caused by the following mechanism : Inhibition of Ca^{+} influx into the myocardial cell as a result of both decrease in the extracellular Ca^{+} concentration and inhibition of ATP production and the Na^{+}-Ca^{+} exchange.

Introduction

Sodium fluoride (NaF) is a medicine available for prevention of dental caries. However, accidental over-ingestion can occur, causing severe acute toxicity. Haynes and Murad described that signs of acute fluoride poisoning which the patient shows are : increasing irritability of the nervous system including paresthesias, positive Chvostek sign, hyperactive reflexes, and tonic and clonic convulsions, they also stated that these signs are related to the calcium-binding effect of fluoride (1).

NaF is a well-known inhibitor of anaerobic glycolysis. In the presence of Mg^{+} and phosphate, fluoride ions strongly inhibit an enolase which catalyzes a reaction in the glycolytic sequence (2). Muscle contraction requires the energy released by the breakdown of adenosine triphosphate (ATP) which was produced in glycolysis (3). Therefore it is thought that this property of NaF may participate in the inhibition of heart movement.

It is of great interest that NaF acts on the contraction of both skeletal muscle and intestinal smooth muscle augmentatively, but on that of cardiac muscle decreasingly. Anticurare action of NaF has been demonstrated by Koketsu and Gerard by means of frog neuromuscular junction (4). Suzuki reported that small doses of NaF promoted the movement of the rabbit intestine, but it

inhibited the frog heart motility (5). These phenomena caused by NaF suggest the possibility of NaF to be a cholinergic agonist (6-8).

In order to clarify the mechanism of inhibition of myocardial contraction by NaF, I compared the actions of parasympathomimetic agent, decalcifying drugs and metabolic inhibitors with those of NaF respectively and in addition we investigated the effect of NaF on the Na^+-Ca^{++} exchange mechanism related to the intracellular Ca^{++} concentration.

Materials and methods

Materials used in this experiment were the isolated hearts of bullfrog *Rana catesbeiana* weighing about 200g. Two methods introduced were as follows:

I) Yagi's cannula was used to perfuse a whole heart (9). The perfusion apparatus has the function that the perfusate which flows out of aorta returns to the cannula connected to posterior vena cava to circulate through the heart. Details are described in the reference (9). The isometric tension of autonomic heart contraction and the heart rate were measured simultaneously by means of a polygraph (San-ei sokki: 141-6) mediated through the force-displacement transducer (Nihon kohden: SB-1T). Test drugs were added to the perfusate (Ringer's solution) in vena cava cannula.

II) Strips were prepared from the auricle, about 2mm in width and 15 mm in length. Both the response to drugs and the recovery of the auricular muscle were so fast that the auricle was introduced as a material. The strip was mounted horizontally in an acryl chamber (12×48×12 mm) containing 3 ml perfusate. The chamber was always perfused with Ringer's solution by means of a peristaltic pump (Harvard apparatus: 1201, perfusion rate: 12 ml/min). A pair of platinum wire electrodes (0.5 mm in diameter and 15 mm in length) were placed on the opposite walls of the chamber. Twitch was caused by field stimulation (voltage: 10 V, duration: 10 msec, frequency: 0.5 Hz, wave form: rectangular single pulse). Ca^{++}-free, Ca^{++}-deficient or Ca^{++}-excess solutions (0-2.2 mM) were prepared by reducing or increasing $CaCl_2$ concentration in normal Ringer's solution. Na^+-deficient contracture was generated by perfusion with the low-Na^+ Ringer's solution which was made by replacing all of NaCl with choline chloride (118 mM) (10). Ca^{++} concentration in low-Ca^{++} Ringer's solution, which was lowered by adding NaF to normal Ringer, was measured with an atomic absorption photometer (Shimadzu: AA-640, wave length: 4226.7 Å) after centrifugating (3000 rpm, 30 min) to remove CaF_2 produced as a result of reaction of NaF upon $CaCl_2$. In the case of NaF application it was added to the perfusate in which NaCl concentration equivalent to applied NaF was reduced in order to keep the isotonicity. Contractile tensions were measured the same as described in method I).

The composition of normal Ringer's solution was as follows (in mM/liter): NaCl,110; KCl, 1.9; $CaCl_2$, 1.1; NaH_2PO_4, 0.5; $NaHCO_3$, 2.4 and glucose, 5.6. The pH was adjusted to 7.3. Test drugs were NaF (Wako Pure Chem.), acetylcholine chloride (Ach, Daiichi Pharmaceut.), atropine sulfate (Tanabe Pharmaceut.), ethylene diamine tetraacetic acid (EDTA, Nakarai Chem.), ATP (Wako Pure Chem.), iodo acetic acid (IAA, Nakarai Chem.), sodium oxalate (Nakarai Chem.), 2, 4-dinitrophenol (DNP, Nakarai Chem.), adrenaline (Daiichi Pharmaceut.) and theophylline (Nakarai Chem.). All experiments were performed at room temperature (20-25°C).

Results

I) NaF (10 mM) decreased heart contractile tension to about 70% 2 min after application compared with the control state and slightly reduced the heart rate after the transient rise. Although Ach (10^{-9}g/ml) inhibited the contraction more intensively than 10 mM NaF, it showed no inhibition under pretreatment of atropine sulfate (10^{-5}g/ml). On the other hand atropine had no effect on the inhibitory action of 10 mM NaF (Fig. 1.). 2-Ca^{++}Ringer's solution which contains two-fold Ca^{++} concentration as high as normal Ringer weakened

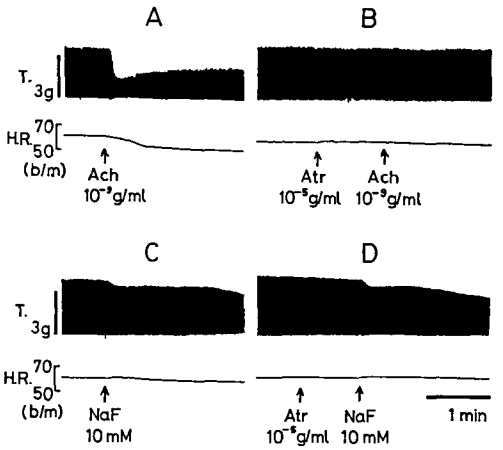

Fig. 1. The effect of atropine sulfate (Atr) on the inhibitory action of Ach or NaF on the autonomic movement of frog heart. Atropine antagonized the action of Ach completely (A,B) but it had no effect on that of NaF (C,D). T: tension, H.R.: heart rate (b/m: beats per min.)

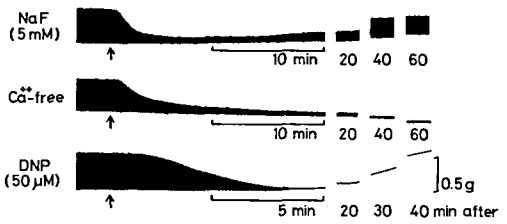

Fig. 3. The effect of long-term application of each one of these NaF, Ca^{++}-free Ringer's solution and DNP on the myocardial twitch. The twitch tension was decreased in all experiments. The Ca^{++}-free Ringer decreased the resting tension. On the contrary NaF raised it, which was resemble to that in the case of DNP application.

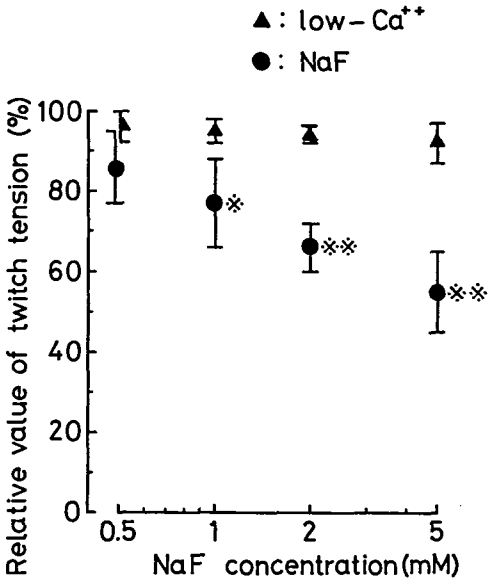

Fig. 2. Comparison of the action of NaF with that of low-Ca^{++} Ringer's solution on the twitch of auricular muscle. NaF decreased the twitch tension more intensively than the low-Ca^{++} Ringer. Twitch tension is given as a relative value of tension 3 min after treatment to that before treatment. Each point represents the mean value obtained from 5 experiments and vertical bars S.D. Significance of difference to the group treated with the low-Ca^{++} Ringer: ※ $P<0.05$, ※※ $P<0.01$.

the action of 10 mM NaF as well as EDTA (4×10^{-5}g/ml), a Ca^{++} -chelating drug. I examined the effect of 10 mM NaCl as a control experiment. Only a transient inhibition was observed immediately after the application. Therefore the inhibition by NaF may not be due to hypertonicity but to fluoride ions.

II) The action of sodium oxalate, a decalcifying drug, was tested on the twitch of auricular strips. NaF (2 mM) inhibited the twitch more strongly than 1 mM sodium oxalate equivalent to 2 mM NaF ($p<0.01$). The actions of NaF (0.5-5 mM) on the twitch tension were compared with those of low-Ca^{++} Ringer's solution 3 min after the application. NaF inhibited the twitch more intensively than the low-Ca^{++} Ringer (Fig.2).

The effect of long-term application of 5 mM NaF was compared with that of Ca^{++}-free Ringer's solution or 50μM DNP. The twitch tension was decreased in all experiments. Though the resting tension was decreased by the Ca^{++}-free Ringer, it was raised by either NaF or DNP. It was very interesting that the Ca^{++}-free Ringer and DNP inhibited the twitch step by step until the end but only NaF initially inhibited it strongly and then the twitch tended to recover gradually (Fig. 3).

The effect of 1.5 μM ATP on the inhibitory actions of NaF (0.5-5 mM) were investigated by adding it to NaF-containing Ringer's solution. ATP markedly antagonized the action of NaF whose concentration was lower than 2 mM (Fig. 4.)

Interactions of 2 mM NaF were investigated respectively with 10 μM DNP (metabolic inhibitor), 0.2 mM IAA (glycolysis blocker), 2.5 μM adrenaline (adenyl cyclase activator)

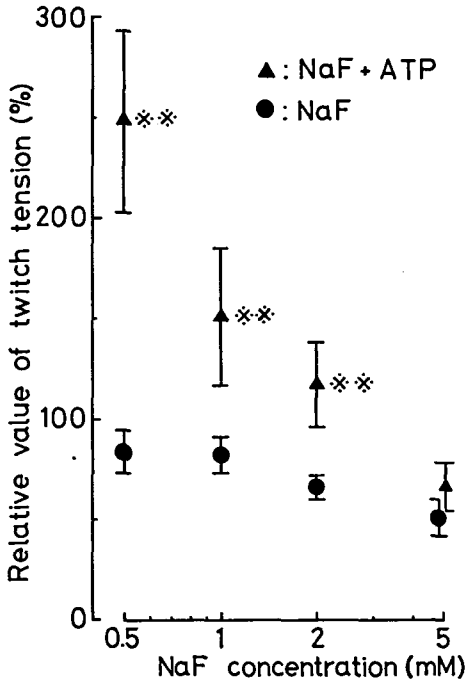

Fig. 4. Effects of ATP (1.5 μM) on the inhibitory action of NaF. ATP markedly weakened the action of NaF. Each point represents the same as in Fig. 2.

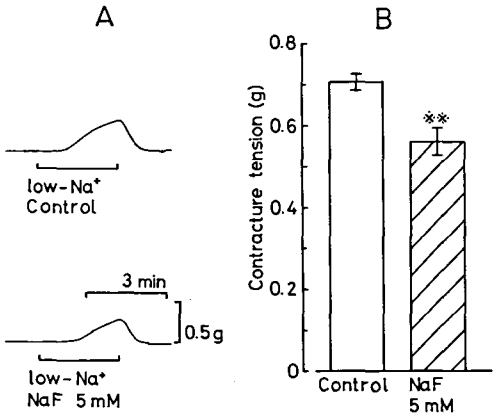

Fig. 5. The effect of NaF on the Na^{+}-deficient contracture. NaF decreased the contracture tension (A). Each column represents the mean value obtained from 5 experiments and vertical bars S. D. (B). Significantly different from control, $p<0.01$.

and 0.1 mM theophylline (phosphodiesterase inhibitor). DNP and IAA further promoted the inhibitory action of NaF on the twitch but adrenaline and theophylline weakened it.

When the Na^+ concentration in Ringer's solution was lowered, the Na^+-deficient contracture was caused by increase of Ca^{++} influx mediated through the Na^+-Ca^{++} exchange mechanism (10). NaF (5 mM) significantly inhibited the Na^+-deficient contracture in comparison with the control experiment in which 5 mM NaCl was applied (Fig. 5).

Discussion

NaF shows several kinds of properties on a living cell, for example, decalcification, metabolic inhibition, enzyme inhibition *etc*(1). And there is some evidence that NaF has an effect similar to the excitement of cholinergic nerves. It was reported that NaF augmented the contractile movement of skeletal muscle and smooth muscle but it depressed that of cardiac muscle (4-8). Thus the antagonism of atropine, a parasympatholytic drug, to the effect of NaF was investigated. If NaF mainly exerts the effect as a parasympathomimetic drug on the heart, its inhibitory action as well as that of Ach must be distinguished or at least weakened by pretreatment of atropine. But in this experiment atropine had no effect on the action of NaF. It is not likely that the inhibition of heart contraction by NaF is based on the excitation of vagus nerve or its effector.

Since pioneering experiment of Ringer it is well known that external Ca^{++} is essential for contractile activity of cardiac muscle (11) and it is especially important for frogs which provide no transverse tubular system and poorly a sarcoplasmic reticulum in myocardial cells (12). The action of NaF was compared with that of a Ca^{++}-chelating drug. When 2-Ca^{++} Ringer's solution was used as a perfusate, the effect of NaF was resemble to that of EDTA, that is, the 2-Ca^{++} Ringer interfered with the inhibition by NaF. This result suggests that the inhibition of myocardial contraction has reference to the reduction of Ca^{++} concentration in the perfusate. This is supported by the report of Tamura *et al.*, i. e., extracellular Ca^{++} formed relatively insoluble salt, CaF_2, in the presence of NaF and thus it caused a deficiency of extracellular Ca^{++} concentration, which might decrease the myocardial contractile force (13). But considering a subsequent investigation the inhibition is not always thought to be caused by only decrease of extracellular Ca^{++} concentration, because NaF inhibited the twitch more strongly than either sodium oxalate equivalent to NaF or the low-Ca^{++} Ringer which contains the Ca^{++} concentration as low as that in case of NaF application to normal one. The other factors also must take part in the twitch inhibition by NaF.

It was reported that cyclic 3', 5'-adenosine monophosphate (cyclic AMP) augmented the myocardial contraction (14, 15). Cyclic AMP activates a protein kinase which catalyzes the phosphorylation reaction at the inside of cell membrane, at a troponine of contractile proteins and at the membrane of sarcoplasmic reticulum (16-18). Therefore cyclic AMP increases the amplitude of slow Ca^{++}inward current, raises the affinity for Ca^{++} and accelerates the Ca^{++} pump respectively. Cyclic AMP is produced from ATP under catalysis by adenyl cyclase. Accordingly, it is thought that NaF may inhibit the twitch dominantly by causing a reduction of ATP(19,20) which is a substrate of adenyl cyclase, while NaF activates the enzyme (21). The rise of resting tension caused by the long-term application of NaF may be a rigor-like state in muscle which is generated by inhibition of the plasticizing effect of ATP (22), because it was hardly restored by wash out with normal Ringer's solution.

Ca^{++} fluxes across the myocardial cell membrane may also occur in exchange for Na^+ (23) via

a Na^+-Ca^{++} carrier mechanism separate from the slow channel. The magnitude of Ca^{++} flux is dependent on the relative intracellular and extracellular Na^+ concentration (24, 25). If the extracellular Na^+ concentration is reduced, Ca^{++} flows into the cell and causes the Na^+-deficient contracture (10). In this experiment NaF markedly decreased the tension of Na^+-deficient contracture. This phenomenon suggests that Na^+-Ca^{++} exchange perhaps may be inhibited by NaF. NaF may reduce the Ca^{++} influx as a result of inhibiting the carrier of Na^+ and Ca^{++}or indirectly decreasing the affinity of the carrier for Na^+ or Ca^{++}, or both by inhibition of ATP production (26).

In conclusion, observations described above imply that NaF may not only reduce the extracellular Ca^{++} concentration but also inhibit the production of ATP, and may consequently decrease the Ca^{++} influx mediated by both a slow channel and the Na^+-Ca^{++} exchange mechanism and thus NaF may weaken the force of myocardial contraction.

As another mode of inhibition by NaF it may block the sympathetic nerves but it is not clear in this study.

The effect of long-term application of NaF, that is, tendency to recovery following inhibition is similar to symptoms of the acute poisoning of a small dose (5) and its mechanism is a subject of absorbing interest. Perhaps it may be related to the recovery that NaF as well as ouabain, a cardiac glycoside, inhibits the Na^+-K^+ ATP ase(27).

In order to investigate in greater detail the effect of NaF on the transmembrane Ca^{++} flux it will be necessary to introduce some other methods. Electrophysiological techniques may be effective.

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