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ELECTROPHYSIOLOGICAL EFFECTS OF THE SALICYLATES ON ISOLATED ATRIAL MUSCLE OF THE RABBIT

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1 Intracellular recordings were made from cells of the sinoatrial (S-A) node region and from atrial muscle fibres of rabbit hearts. The effects of sodium salicylate and 5-bromo salicylate on various parameters of the membrane action potential were studied.

2 5-Bromo salicylate (30–100 μM) and sodium salicylate (300–500 μM) caused a dose-dependent decrease in the frequency of discharge of the SA node cells. Applications of atropine (2.6 μM) with propranolol (3.3 μM) did not affect the negative chronotropic effect, whereas adrenaline (5 μM) reversed it.

3 Depolarization and shortening of the action potential duration were found in atrial muscle fibres after the application of 5-bromo salicylate (60–100 μM). The reduction of the action potential duration (APD) was not affected by atropine (2.6 μM).

4 Higher concentrations of 5-bromo salicylate (> 100 μM) also caused a dose-dependent reduction in the action potential amplitude (APA), in the overshoot (OS) of the action potential and in the maximum rate of rise of the action potential (V_{max}). All these effects were completely reversed on washing.

5 Substitution of the NaCl of the bathing Tyrode solution by an equimolar concentration of Na isethionate did not affect the plateau depression induced by the salicylates in atrial muscle fibres.

6 After increasing the K concentration to 27 mM in the presence of isoprenaline (1 μM), 'slow responses' were obtained upon stimulation. 5-Bromo salicylate (20–60 μM) and sodium salicylate (100 μM) decreased reversibly the amplitude and the rate of rise of the 'slow response'.

7 A four fold increase in Ca concentration of the standard Tyrode solution did not antagonize the plateau depression of atrial muscle fibres or the negative chronotropism induced by salicylates.

8 Addition of CsCl (10 mM) to the Tyrode solution did not affect the shortening of the APD induced by the salicylates in atrial muscle fibres.

9 When the K concentration in the Tyrode solution was increased from 2.7 mM to 5.4 mM, the effects of 5-bromo salicylate on the APA, OS and V_{max} were potentiated. However, a significant reduction in the shortening of the APD produced by the salicylate was observed.

10 It is suggested that the salicylates possibly depress the slow inward current in both S-A node cells and atrial muscle fibres of the rabbit heart. In atrial muscle fibres, a concomitant increase in the outward potassium current is probably involved.

Introduction

The salicylates have recently been used as a tool in the investigation of the electrophysiology of excitable tissues since it is believed that they can affect the surface electrical potential of the membranes (McLaughlin, 1973; Cohen, Noble, Ohba & Ojeda, 1979a, b; Atwell, Bergman & Ojeda, 1979).

Whether acting through a modification of surface charges or not, salicylates do affect the ionic permeabilities of the membranes and their effects have been studied in detail. In molluscan neurones, Barker & Levitan (1971) found an increase in K permeability and a decrease of Cl permeability after the application of concentrations of sodium salicylate producing an increase in membrane potential and a decrease in membrane resistance. In squid isolated axons, Riccioppo Neto & Narahashi (1976) with the aid of voltage clamp techniques, described a block of both peak transient sodium conductance and steady state potassium conductance. In mammalian non-myelinated nerve fibres, Riccioppo Neto (1980) observed a frequency-dependent decrease of the C-fibre action potential amplitude and a block of the electrogenic component of the sodium-potassium pump. In sheep cardiac Purkinje fibres, Cohen *et al.*

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(1979a) described an increase followed by a decrease of the resting membrane potential, a prolongation of the action potential and shifts in the hyperpolarizing direction of the activation curve for the pacemaker current i_{k2} and of the apparent reversal potential for i_{k2}.

In the present work the actions of salicylates have been studied on the membrane resting and action potentials of sinoatrial and atrial muscle fibres of the rabbit heart and an attempt has been made to correlate these actions with alterations in the ionic permeabilities underlying the electrical phenomena.

Methods

Rabbits (2.5–3.5 kg) were stunned by a blow on the head and exsanguinated by cutting their carotid arteries. Their hearts were quickly excised, dissected in cool Tyrode solution and transferred to the Sylgard-lined bottom of a 15 ml tissue chamber.

Tyrode solution, aerated with 95% O_2 and 5% CO_2 (pH 7.4) flowed over the preparation at a rate of 6–7 ml/min and at a temperature of 36 ± 0.5°C. Right atrial preparations included the sinoatrial nodal area and beat spontaneously. Quiescent preparations were prepared from the opened out left atrial appendage. Experimentation was started after a 60–90 min period of adaptation to the new conditions.

When necessary, square wave pulses from a Grass stimulator (S4-SIU4) and delivered through a pair of Teflon coated silver wire electrodes were used. The basic drive stimulus (1.5–2 × threshold and 1 ms duration) was delivered, unless otherwise stated, at a rate of 1 Hz.

Transmembrane potentials were recorded (at a distance < 3 mm from the stimulation points) with conventional glass microelectrodes filled with 3 M KCl having d.c. resistances of 10–30 MΩ. The output from the microelectrode was fed to an oscilloscope (Tektronix 5112) via Ag-AgCl connections and an input capacity neutralization preamplifier (Grass P-16). The maximum rate of rise of the action potential (V_{max}) was determined electronically using an OP-AMP (Analog Devices 118 A) and a RC circuit with time-constant of 50 μs. The output of the differentiator was linear with rates of potential change in the range of 50–1000 V/s. Graphical differention was also made in those cases in which V_{max} was below 50 V/s by measuring the slope of a straight line drawn by eye through the steepest part of the upstroke. For analysis of the sinoatrial rate, bipolar surface electrograms were recorded from the *crista terminalis* and the signal was simultaneously displayed on the oscilloscope. Oscilloscope traces were photographed on 35 mm film with a camera (Nihon-Kohden PC-3A). Changes in resting potential were simultaneously recorded on a servo-driven potentiometric pen recorder. The photographical records were enlarged (7 ×) and the following parameters were measured: maximum diastolic potential (MDP), action potential amplitude (APA), overshoot of action potential, maximum upstroke velocity of action potential (V_{max}), duration of action potential from its peak to 50% (APD_{50}) and 90% (APD_{90}) repolarization, and sinoatrial rate (SAR). Except in conditions in which a high calcium Tyrode solution was used, only recordings obtained from impalements that were maintained in an individual cell throughout the application of a determined concentration of salicylate (around 30 min) are described.

Tyrode solution served as normal perfusion fluid and was of the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.45, $NaHCO_3$ 12, NaH_2PO_4 0.32 and glucose 5.5. 'Low chloride' solution was prepared by replacing NaCl with equimolar sodium isethionate. In order to obtain the 'slow response' (Carmeliet & Vereecke, 1969; Pappano, 1970), an equimolar concentration of NaCl was substituted by KCl (27 mM); isoprenaline (1 μM) was added to the superfusion fluid and the preparations were stimulated at a frequency of 0.1 Hz.

Drugs used were: atropine sulphate (Merck); (−)-isoprenaline hydrochloride (Sigma); (−)-adrenaline (Sigma); propranolol hydrochloride (I.C.I.); caesium chloride (Sigma); 5-bromo salicylic acid (Aldrich);

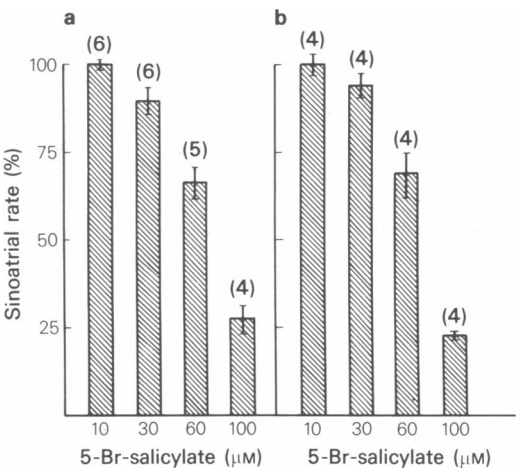

Figure 1 Effects of 5-bromo salicylate on the frequency of discharge of the rabbit S-A node. The frequency was measured 30 min after the application of the drug except for 100 μM, in which the frequency was measured 20–25 min after the beginning of the superfusion. In (b) the addition of the salicylate was preceded (10 min) and followed by superfusion of the node with Tyrode solution containing both atropine (2.6 μM) and propranolol (3.3 μM).

Table 1 Effects of 5-bromo salicylate on parameters of the action potential recorded in the sinoatrial region of 4 rabbit hearts

Conc. (μM)	*MDP* (mV)	*APA* (mV)	*OS* (mV)	APD_{50} (ms)	APD_{90} (ms)
0	68.0±2.4	69.3±2.7	1.3±0.4	60.3±1.9	119.0±2.9
30	67.8±2.7	68.5±2.3	0.7±0.6	60.5±1.8	118.5±1.7
0	63.5±1.8	66.3±0.7	2.8±1.8	58.5±1.0	113.3±3.2
60	61.8±1.6	64.5±0.7	2.7±1.6	58.0±1.4	113.7±3.1
0	70.0±2.7	72.8±2.6	2.8±0.5	59.5±1.3	109.0±3.2
100§	65.5±2.6*	60.5±2.1**	−5.0±1.0**	58.5±1.7	110.0±2.0

*$P<0.02$; **$P<0.01$; paired Student's t test; § Measured during the first 15–20 min of drug superfusion.

salicylic acid (Aldrich). The sodium salts of the salicylates were obtained according to Levitan & Barker (1972). Different concentrations of the salicylates were applied at random and the preparations were always washed free of drug for 60 min between the applications. Data are expressed as mean values ± s.e.mean.

Statistical analysis of measured parameters was performed by Student's t test and P values of less than 0.05 were considered to indicate significant differences. More details of each procedure are given below.

Results

Effects of the salicylates on the sinoatrial node

Superfusion of the sinoatrial region with 5-bromo salicylate in concentrations ranging from 30 μM to 300 μM reduced the sinus rate in all experiments (Figure 1) which was unaffected by a combination of atropine (2.6 μM) and propranolol (3.3 μM) (Figure 1). Concentrations of 100 μM or greater stopped the spontaneous beating of the sinoatrial node completely and induced a reduction of the MDP. As shown in Figure 2 and in Table 1 there was a reduction in the rate of diastolic depolarization concomitant with a reduction in APA, but no alteration of the APD_{50} or APD_{90} in the range of concentration used.

In three experiments the negative chronotropic effect of 5-bromo salicylate (60 μM) was immediately reversed by superfusion with Tyrode solution containing 5-bromo salicylate (60 μM) and adrenaline (5 μM). However, adrenaline did not restore the rhythm of a heart stopped by a greater concentration of the salicylate (100 μM; $n=3$). In all circumstances in which adrenaline was perfused together with the salicylate, the sinus rate discharge increased greatly during the first 5 min following the washing of the preparation with drug-free Tyrode solution. In three experiments the effects of 300 μM sodium salicylate were nearly equivalent to those induced by 60 μM 5-bromo salicylate. All these effects disappeared completely in about 30–40 min of superfusion with drug-free Tyrode solution.

Effects of 5-bromo salicylate on atrial muscle fibres

Up to the concentration of 100 μM the most striking effect, after a period of equilibration of 30 min, was a reduction in the total APD (Figure 3) without significant changes in APA, V_{max} and in the overshoot.

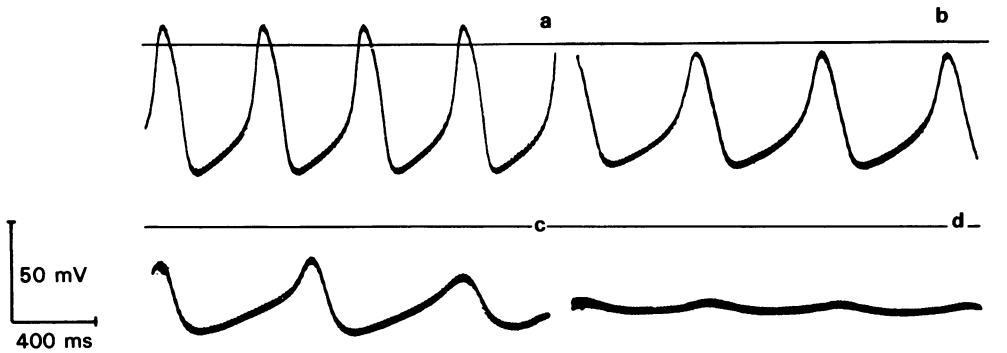

Figure 2 Intracellular recording of the effect of 5-bromo salicylate (100 μM) on the spontaneous rate and action potentials of a S-A node cell. (a) Control; (b), (c) and (d), 18, 25 and 30 min after addition of the drug. Upper trace: reference line; lower trace: intracellular recording.

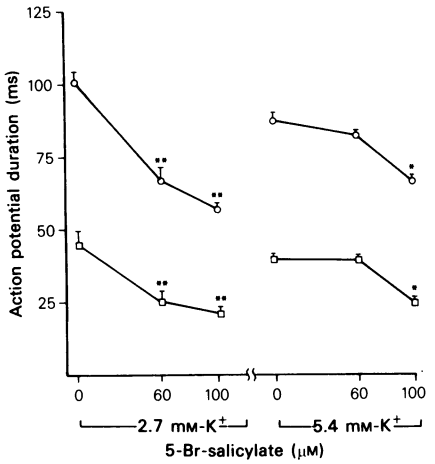

Figure 3 Effects of 5-bromo salicylate on the action potential duration (APD) of atrial muscle fibres superfused with Tyrode solution containing different K concentrations. APD_{50} (□) and APD_{90} (○): durations measured at 50% and 90% repolarization. (*$P<0.02$; **$P<0.01$; paired Student's t test.

The MDP underwent a gradual depolarization in the majority of the preparations. The application of 300 μM salicylate for 15 min decreased MDP, APA and V_{max} by 24±7%, 29±4% and 47±8% of their control magnitudes, respectively. A period of total inexcitability always followed the application of concentrations greater than 200 μM. In all preparations studied a negative inotropism was clearly noted through microscopic observation during applications of concentrations around 60 μM or greater.

In three experiments, the addition of atropine (2.6 μM) to the superfusion fluid did not affect the acceleration of the repolarization phase induced by 5-bromo salicylate (100 μM). All the effects described above were also readily reversible upon washing.

Effects of reducing the chloride concentration

Superfusion with sodium isethionate 'low chloride' solution increased the action potential duration of the atrial preparations. The APD_{50} and APD_{90} of 4 experiments were 53.3±3.2 ms and 103.8±2.4 ms, respectively. The addition of 5-bromo salicylate at two different concentrations still induced the shortening of the action potential duration found in normal chloride perfused hearts. The APD_{50} and APD_{90} were, respectively, 27.8±5.3 ms and 63.5±6.6 ms 30 min after the application of 60 μM 5-bromo salicylate and 24.8±3.9 ms and 62.8±4.5 ms, 30 min after 100 μM.

Effects on the 'slow response'

After increasing the potassium concentration to 27 mM in the presence of isoprenaline (1 μM), the resting membrane potential of the atrial muscle fibres fell to 45.3±0.3 mV (44 fibres impaled in 4 preparations). In the presence of either, low concentrations of 5-bromo salicylate (30–100 μM; 4 experiments) or of a higher concentration of sodium salicylate (100 μM; 3 experiment), there was a gradual decrease of the amplitude of the 'slow response' which was accompanied by an acceleration of the repolarization phase. Within 25–30 min of drug application the 'slow response' was completely suppressed (Figure 4), an effect which was reversible upon washing for 20–30 min with drug-free Tyrode solution.

Small doses of 5-bromo salicylate (20 μM; 3 experiments) induced only a decrease in V_{max}; the smaller concentration of 15 μM did not affect the slow response in another 2 experiments. A rather large depression of contractility and, occasionally, asystole was observed during the equilibration period.

Effects of an increase in the extracellular calcium concentration

The calcium concentration was increased from 1.8 mM to 7.2 mM by the addition of $CaCl_2$ to the standard Tyrode solution. The changes induced on S-A nodal action potentials were similar to those described by Seifen, Schaer & Marshall (1964). Following a period of equilibration (15 min in high-calcium Tyrode solution) 10–15 cells of each of 4 hearts studied were impaled in order to obtain control data. After superfusion with 5-bromo salicylate (60 μM for a period of 30–35 min) some 10–15 cells were again impaled. There was a mean fall of 40%±3 in the sinus discharge of the 4 hearts studied.

The most important effects induced by the increase in the calcium concentration on the action potential configuration of atrial muscle fibres were an elevation of the plateau height, a shortening of APD_{50} and a slight prolongation of APD_{90}. The procedure for obtaining data from 4 auricular appendages was similar to that outlined above. An increase in extracellular calcium concentration was not completely effective in antagonizing the reduction in APD induced by the salicylate (Table 2).

Influence of increased potassium concentration on the effects of 5-bromo salicylate

There were statistically significant reductions ($P<0.01$) of APA (28±3%), V_{max} (36±9%) and of the overshoot (39±3%) in four preparations bathed with Tyrode solution containing 5.4 mM potassium and treated by 5-bromo salicylate

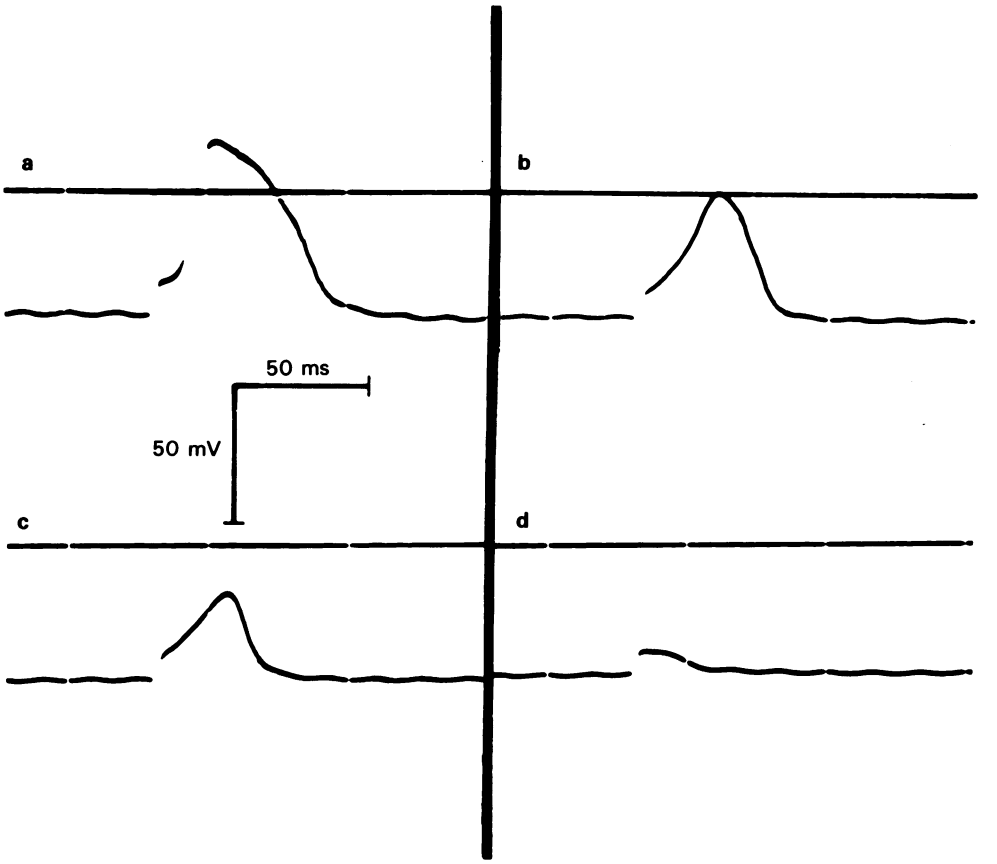

Figure 4 'Slow-responses' obtained in K^+-depolarized, isoprenaline-treated atrial muscle fibres. (a) Control; (b), (c) and (d): 15, 20 and 25 min, respectively, during superfusion with 5-bromo salicylate (60 μM). Upper trace: reference line; lower trace: intracellular recording.

(100 μM) for 30 min. In these preparations the MDP fell from 76.0±2.0 mV to 75.3±0.5 mV after the application of the salicylate. However, as shown in Figure 3 the APD shortening was significantly reduced by increasing the potassium concentration.

Effects of 5-bromo salicylate in Tyrode solution containing caesium

The addition of CsCl (20 mM; three preparations) to the Tyrode solution bathing the atrial muscle fibres

Table 2 Effects of 5-bromo salicylate on the action potential parameters of atrial muscle fibres of 4 hearts superfused with high Ca^{2+} (7.2 mM/l) Tyrode solution

Conc. (μM)	n	*MDP* (mV)	*APA* (mV)	*OS* (mV)	APD_{50} (ms)	APD_{90} (ms)	V_{max} (V/s)
0	28	80.1±0.8	113.0±1.0	32.9±1.0	25.4±0.4	108.6±0.8	157.8±1.8
60	27	78.0±0.8	110.7±1.4	32.7±1.5	24.5±0.4	96.3±1.3*	154.4±1.5
0	22	79.2±0.8	109.6±1.6	30.4±1.7	23.8±0.5	110.0±1.1	148.6±3.9
100	23	77.8±1.0	108.0±1.4	30.2±1.7	12.6±0.4*	62.2±0.7*	141.5±4.8

n = number of observations; *$P<0.01$; Student's t test for the difference of means.

caused a rapid depolarization (of about 35 mV) and a complete loss of excitability. A smaller concentration of caesium (10 mM; three preparations) induced a depolarization of 22 ± 1.2 mV. Under these conditions, 5-bromo salicylate (100 μM) was still capable of inducing a further reduction in APD_{50} and APD_{90} of $45 \pm 2\%$ and $31 \pm 1\%$, respectively.

Discussion

The pronounced effects of salicylates on isolated atrial muscle fibres of the rabbit were depolarization and a marked decrease in APD. On sinoatrial fibres there was a decrease in spontaneous frequency, depression of the APA and depolarization.

On sheep cardiac Purkinje fibres Cohen *et al.* (1979a) found variable effects of sodium salicylate (10–50 mM) on resting membrane potential. There was usually an initial hyperpolarization followed by depolarization; sometimes only depolarization was noted. There was also an increase in APD and reduced pacemaker depolarization.

Reduction of the APD of cardiac fibres has also been described after the application of metabolic inhibitors such as dinitrophenol (Kleinfeld, Magin & Stein, 1961; Haas, Kern & Einwächter, 1970; McDonald & MacLeod, 1973), NaCN and iodoacetate (MacFarlane, 1960). Using voltage clamp techniques, Haas *et al.* (1970) observed a great increase in the outward current of frog cardiac fibres, but they did not identify it with a K current, since K efflux did not show a similar change.

In our experiments the shortening of the APD caused by salicylate was not influenced by replacement of chloride with isethionate; this rules out the possibility of an increased gCl underlying the phenomenon. On the other hand, an increase in the extracellular K concentration reduced, but did not block, the salicylate-induced shortening of the APD. This suggests an increase in gK by salicylate (Hall & Noble, 1963; Noble, 1965). Indeed, an increase of gK in membranes of neurones of *Navax inermis* has been found by Barker & Levitan (1971) after application of salicylates. On the other hand, in the presence of caesium, which has been shown by Isenberg (1976) to block completely the pacemaker current i_{k2} and the instantaneous time-independent current i_{k1} in cardiac Purkinje fibres, the application of 5-bromo salicylate still shortened the APD. If the concentration of Cs used in our experiments was sufficient to block g_{k1}, we are left with the possibility that the component i_x of the outward current is the one involved in the action of salicylate. This is also in agreement with the results obtained by Cohen *et al.* (1979b) in the guinea-pig ventricle and substantiates the view that i_x is the main component in determining the APD in rabbit atrial muscle fibres. Similar conclusions have been drawn from studies involving cat and guinea-pig ventricular muscle (Katzung & Morgenstern, 1977; MacDonald & Trautwein, 1978).

APD is also shortened by a direct reduction of the slow inward current (Paes de Carvalho, Hoffman & Paula Carvalho, 1969; Wit & Cranefield, 1975; Eick, Nawrath, McDonald & Trautwein, 1978). The suppression of the 'slow response' that we observed indicates that this reduction occurs even with concentrations of salicylate insufficient to reduce the other parameters of the cardiac action potential such as APA, MDP, V_{max} and the overshoot.

Possibly both phenomena (a decrease in the slow inward current and an increase in an outward current) account for the diminished plateau during salicylate action. These 'direct' and 'indirect' effects (Eick *et al.*, 1978) would also explain the decreased contractility found in the muscle fibres treated with salicylate (Blood, 1977) and the relative inefficacy of calcium ions in antagonizing the effect in the plateau duration. Whatever the ultimate mechanism might be, the acceleration of the repolarization phase was shown to be independent of the activation of a muscarinic receptor since it was not abolished by atropine.

A reduction of the fast inward sodium current during phase O of the atrial action potential, as already demonstrated for squid axons (Riccioppo Neto & Narahashi, 1976) and the depolarization observed when higher concentrations of 5-bromo salicylate ($> 100\,\mu$M) were applied on preparations bathed in 2.7 mM potassium, could be responsible for the reduction of the APA, overshoot and V_{max}. Moreover, in accordance with the well known influence of K concentration on the effects induced by agents that depress phase O of the action potential (Singh, 1971; Singh & Vaughan Williams, 1971), we observed a facilitation of the effects of salicylate on APA, V_{max} and the overshoot in the presence of 5.4 mM potassium.

Depressor effects on the sinoatrial discharges should be expected following the results of salicylate blockade of the 'slow response' in atrial muscle. It has been shown that the slow inward current plays an important role in the slow diastolic depolarization and in the upstroke of the action potential of sinoatrial cells (Brown & Di Francesco, 1980; Noma, Kotake & Irisawa, 1980). Furthermore, agents such as verapamil which inhibit the slow inward current directly, depress sinus node discharge (Zipes & Fischer, 1974; Wit & Cranefield, 1975). The negative chronotropic effect induced by the salicylates does not seem to involve an activation of muscarinic receptors or a blockade of the release of noradrenaline from sympathetic nerve terminals (Irisawa, 1978), since it was

not reduced by a combination of atropine and propranolol. In addition, the adrenaline-induced reversal of the negative chronotropic effect of salicylates is an indication that β-adrenoceptors are freely accessible.

One interesting finding was the absence of an effect of salicylates on the APD_{50} and APD_{90} of sinoatrial cells, whereas these parameters were reduced in K^+-depolarized, isoprenaline-treated atrial muscle fibres (Figure 3). If we assume a close link between slow inward current and outward potassium currents a reduction of both currents should produce only a decrease in APA, as observed after salicylate application. As has already been suggested for ventricular muscle (Bassingthwaighte, Fry & McGuigan, 1976), some of the potassium permeability in the mammalian S-A node is linked with calcium permeability (Brown & Di Francesco, 1980). On the other hand, such possible links should be strongly affected by the salicylates if these agents actually decrease slow inward and increase outward currents in atrial muscle fibres.

Finally, the depolarization found in both types of fibres studied can be attributed to a salicylate-induced block of the sodium-potassium pump (see Smith & Smith, 1966 for a review). The electrogenic Na^+ pump plays an essential role in the maintenance of the membrane potential under physiological conditions (Kurachi, Noma & Irisawa, 1981) and its inhibition has been shown to induce significant depolarization in many cardiac tissues (Glitsch, 1979).

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