Naunyn-Schmiedeberg's Arch. Pharmacol. 307, 9–19 (1979)

Naunyn-Schmiedeberg's
Archives of
Pharmacology

The Relation Between the Current Underlying Pacemaker Activity and Beta-Adrenoceptors in Cardiac Purkinje Fibres: A Study Using Adrenaline, Procaine, Atenolol and Penbutolol

Keitaro Hashimoto*, Otto Hauswirth, Heinz D. Wehner, and Rolf Ziskoven

Physiologisches Institut II, Universität Bonn,
Wilhelmstrasse 31, D-5300 Bonn, Federal Republic of Germany

Summary. The pacemaker current – i_{K2} – in cardiac Purkinje fibres was analysed using the voltage clamp technique described by Deck et al. (1964). (–)-Adrenaline ($5.5 \cdot 10^{-6}$ M) causes the wellknown shift of the Hodkin-Huxley kinetics in the depolarizing direction. Procaine ($7.3 \cdot 10^{-4}$ M) does not cause any further shift of s_∞ in the presence of adrenaline. Atenolol ($3.8 \cdot 10^{-5}$ M) causes a backshift of the kinetics in the negative direction in the presence of adrenaline and procaine. The instantaneous current-voltage relationship ($\overline{i_{K2}}$) is altered neither with adrenaline, nor with procaine or atenolol. The results exclude the possibility that the local anaesthetic side effect of many beta-adrenoceptor blocking agents may be involved in the backshift of the s-kinetics. The voltage dependence of the reciprocals of the time constants is shifted in a similar way as s_∞ by the sympathomimetic or blocking drugs. Following the application of (–)-adrenaline ($5.5 \cdot 10^{-6}$ M) the (–)-isomere of penbutolol (1.7 and $3.5 \cdot 10^{-6}$ M) is about equally effective in shifting the kinetics back as the (+)-isomere ($3.5 \cdot 10^{-5}$ M). In the presence of (–)-adrenaline, the (+)- and (–)-forms of penubutolol cause virtually no change of the instantaneous current-voltage relationship, $\overline{i_{K2}}$. Thus, (–)-adrenaline and (+)- and (–)-penbutolol are aiming for the s-kinetics whose voltage dependence is controlled by the electric field near the i_{K2}-channel of the membrane and do not influence the number of the i_{K2}-channels. These findings suggest that the sympathomimetic or blocking agents influence the s-kinetics of the pacemaker current i_{K2} by altering the electric field; the fully activated current-voltage relationship which is proportional to the number of the open i_{K2}-channels is not subject to any appreciable modification. The results conclusively show that the kinetics of the pacemaker current can be controlled by beta-adrenoceptors.

Key words: Pacemaker current – Beta-adrenoceptors – Voltage clamp analysis.

Send offprint requests to O. Hauswirth at the above address
* Present address: Department of Pharmacology, University of Niigata, Niigata, Japan

Introduction

The pacemaker potential in cardiac Purkinje fibres is brought about by decline of slow outward current (Deck et al., 1964; Vassalle, 1966; McAllister and Noble, 1966, 1967; Noble and Tsien, 1968), which allows the non time dependent steady state background current (Peper and Trautwein, 1969) to depolarize the membrane. Noble and Tsien (1968) gave a detailed description of the kinetics and the rectifier properties of this pacemaker current which they designated i_{K2}. This current component is described by two factors:

$$i_{K2} = \overline{i_{K2}} \cdot s \qquad (1)$$

where $\overline{i_{K2}}$ is the instantaneous current-voltage relationship depending on voltage only and showing inward going rectification with a marked negative slope in the region positive to about – 70 mV (Noble and Tsien, 1968). s is a dimensionless variable analogous to n (Hodgkin and Huxley, 1952) which controls the degree of activation and which follows from a chemical reaction

$$[1-s] \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} [s].$$

$[s]$ is described under the assumption of a first order kinetic:

$$\frac{ds}{dt} = \alpha_s \cdot (1-s) - \beta_s \cdot s. \qquad (2)$$

0028-1298/79/0307/0009/$02.20

α_s and β_s are rate constants which are voltage dependent only and which empirically have been found to be exponential functions (see Hodgkin and Huxley, 1952; Noble, 1962; McAllister et al., 1975; Tsien, 1974a).

In the specialized conducting tissue (Purkinje fibre) the accelerating effect of adrenaline on the pacemaker potential has been analysed by Hauswirth et al. (1968) and Tsien (1974a, b). It was shown that adrenaline and isoprenaline shift the voltage dependence of the kinetics of i_{K2} in the depolarizing direction (see also Hauswirth et al. 1976b). Likewise it was shown that beta-adrenoceptor blocking agents like pronethalol (10^{-6} M) (Hauswirth et al., 1968) and propranolol (10^{-6} M) (Tsien, 1974a) shift the kinetics back towards their original position. Tsien (1974a) suggested that i_{K2} may be controlled via beta-adrenoceptors since only beta-sympathomimetic compounds like adrenaline and isoprenaline cause a shift of the *s*-kinetics, and since, in striking contrast to this finding, the application of alpha-stimulating drugs did not cause a measurable shift of the kinetics of i_{K2}.

Cranefield et al. (1971) and Giotti et al. (1968, 1973) provided evidence that in Purkinje fibres alpha-receptors are also present. In contrast to the findings of Giotti et al. (1968, 1973), Quadbeck and Reiter (1975) in guinea-pig papillary muscle did not find any participation of alpha-adrenoceptors in the prolonging effect of the action potential caused by noradrenaline (10^{-7} to 10^{-6} M) or isoprenaline (10^{-8} M). From the qualitative resemblence of the effects of noradrenaline (10^{-7} to 10^{-6} M) and isoprenaline (10^{-8} M) both of which were inhibited by propranolol ($5 \cdot 10^{-6}$ M), these authors concluded that the prolongation of the action potential induced by noradrenaline (10^{-7} to 10^{-6} M) or isoprenaline (10^{-8} M) is mediated solely by beta-adrenoceptors. Tsien (1974a) did not find any detectable shift of the steady-state degree of activation of i_{K2} with phenylephrine (10^{-5} M) in the presence of propranolol (10^{-6} M). this suggestion was confirmed by Hauswirth et al. (1976a) in a somewhat extended study using methoxamine ($1.6 \cdot 10^{-4}$ M) a very selective alpha-stimulating agent with beta-adrenoceptor blocking activity (Imai et al., 1961).

However, some of the beta-adrenoceptor blocking agents such as pronethalol and propranolol exert a strong local anaesthetic side effect (Gill and Vaughan-Williams, 1964; Morales Aguilerá and Vaughan-Williams, 1965; Papp and Vaughan-Williams, 1969).

Taking all this information into account the following questions should be answered in voltage clamp experiments:

1. Does the local anaesthetic side effect of beta-blockers play any role in the backshift of the *s*-kinetics?

2. Do beta-blockers without prior administration of adrenaline alter the *s*-kinetics?

3. Do the drugs mentioned above alter the voltage dependence of the kinetics or the instantaneous current-voltage relationship?

4. Does the well known and widely studied stereospecificity of beta-adrenoceptor stimulating and blocking agents hold true also in case of a particular current component, i.e. of a single membrane channel?

In order to answer these questions a full voltage clamp analysis of i_{K2} consisting of the measurements of the fully activated current-voltage relationship, the steady state activation curve and the time and rate constants is required.

Methods

The present experiments were performed in Purkinje fibres of sheep, the approach being similar to the previous work of Noble and Tsien (1968), Hauswirth et al. (1968), Tsien (1974a, b) and Hauswirth et al. (1976a, b). The pacemaker potassium current, i_{K2}, was analysed in preparations which were shorter than one space constant (1 – 2 mm, Weidmann, 1952) using two microelectrode voltage clamp technique (Deck et al. 1964).

Preparations. Sheep hearts were obtained from the slaughter house immediately after sacrifice. After the ventricles were opened and rinsed, the hearts were placed in a cooled (about 8° C) bathing solution. Purkinje fibres were cut out of both ventricles not later than 60 min after slaughter and kept in oxygenated bathing solution at about 35° C. The fibres were given at least 1 h for healing over before they were impaled. The used perspex chamber contained about 0.8 ml solution and was surrounded by aqueous heating fluid which maintained the temperature of the bathing fluid near 35 – 36° C. The bathing solution aerated with 95 % O_2 and 5 % CO_2 flowed from one of several reservoirs continously through the chamber; the exchange of the solution in the bath was quickly performed through a short (30 cm) common tube between the chamber and a tap where the flow could be switched from one reservoir to another. At a flow rate of 1 ml/min the exchange of the bathing solution was 95 % complete after about 180 s.

The composition of the bath solution was the following (mM): Na^+: 148,3; K^+: 4.0; Ca^{2+}: 1.8; Mg^{2+}: 0.5; Cl^-: 144.6; HCO_3^-: 12.0; $H_2PO_4^-$: 0.35; glucose: 15.

(–)-Adrenaline hydrochloride ($5.5 \cdot 10^{-6}$ M) Merck, Darmstadt, Germany) was used as a catecholamine to stimulate beta-adrenoceptors. In order to exclude the possibility that the local anaesthetic side effect of many beta-adrenoceptor blocking agents (Gill and Vaughan-Williams, 1964; Papp and Vaughan-Williams, 1969) might be involved in the backshift of the kinetics of i_{K2}, procaine (novocaine hydrochloride, Hoechst, Frankfurt/Main, Germany) was administered in rather large concentration ($7.3 \cdot 10^{-4}$ M) following the application of adrenaline. Finally, a racemate of atenolol ($38 \cdot 10^{-6}$ M) (Imperial Chemical Industries, Macclesfield, Cheshire, England) was added as a beta-adrenoceptor blocking agent exerting practically no local anaesthetic activity in the concentration used (Hashimoto and Hauswirth, unpublished).

Since only very small amounts of (+)- and (–)-atenolol could be made available to us, we have decided to use an additional beta-blocker, penbutolol (Hoechst, Frankfurt/Main, Germany), whose optical isomeres were available in larger quantities.

In all experiments, the fibres were given about 30 min for equilibration to every new solution before any measurements were obtained. This holds also true in experiments where successively several solutions were administered to the same fibre.

Current and Voltage Recording

Intracellular microelectrodes filled with 3 M KCl which had a d.c. resistence between 10 and 20 mΩ were used for voltage recording and injecting current. Short Purkinje fibres (1 – 2 mm) were impailed with two microelectrodes. The current passing microelectrode was inserted midway between the two cut ends of the preparation. The second microelectrode was impailed about 300 µm apart.

The input stages connected to the intracellular voltage measuring microelectrode and to the reference electrode consisted of integrated circuits with field effect inputs ($R_{in} = 10^{12}\,\Omega$) which are arranged as voltage followers. The transmembrane current was measured by an operational amplifier connected to the bath by an Ag-AgCl half cell electrode. This signal was recorded together with the membrane potential signal on a number of various devices: A R 5103 N storage oscilloscope (Tektronix), a Tektronix 565 oscilloscope where the beam was often photographed with a camera (Grass, Quincy, Mass., U.S.A.), a Brush 240 four channel pen recorder whose frequence response was diminished by 3 dB at 150 Hz and a TR 3200 tape recorder (Bell & Howell, Basingstoke, Hampshire, England) which, in a second run, allowed a signal to be displayed on the oscilloscope or pen recorder with different amplification and time scale than was used in the original experiment. In some experiments, a Schwarzer PEE/4B pen recorder was used whose frequency response was reduced by 3 dB at 300 Hz.

In most experiments, the current signal was additionally filtered by a RC-circuit and displayed on one chart recorder channel at high amplification. The filtering reduced noise but did not affect the measurement of i_{K2} since this current component has a time constant of the order of two seconds in the range of the normal pacemaker potential.

Experimental Procedure

The analysis of the kinetics and rectifier properties of i_{K2} has been extensively described by Noble and Tsien (1968) and Tsien (1974a). Imposing rectangular voltage pulses on the membrane, the steady state degree of activation of i_{K2} (s_∞) was measured as the amplitude of current tails on the return to the holding potential.

The time constant of i_{K2} at potentials positive to the threshold of the excitatory sodium current was measured by progressively shortening the clamp pulses and measuring the amplitudes of current tails following depolarizing clamp pulses (envelope test). The instantaneous current-voltage relationship was obtained by calculating the quotient of the amplitudes of i_{K2} during and following depolarizations to various clamp levels (i_A and i_B; see Fig. 2A inset). This rectifier ratio (i_A/i_B) is then multiplied by the amplitude of s_∞ obtained at the same holding potential resulting in the values of the "rectifier function', $\overline{i_{K2}}$ (Noble and Tsien, 1968).

Possible Errors

a) Voltage Drift. The most obvious source of error is the drift of voltage in this kind of experiments (see Tsien, 1974a). The electronic device caused little drift over several hours – as tested on a model circuit – the most serious source is the tip of the voltage measuring microelectrode (see also Tsien, 1974a). In general, the experiments were performed with continuous impailements of the microelectrodes. In the present experiments the voltage drift somewhat less than 1 mV/h.

b) Voltage Non-Uniformity. The possibility of voltage non-uniformity has been discussed by Tsien (1974a) on the grounds of experimental findings of Deck et al. (1964), Sommer and Johnson (1968), Mobley and Page (1972), McGuigan (1974) and Weidmann (1952) and taking into account theoretical considerations of Jack et al. (1975) and of Johnson and Lieberman (1971). The essence of Tsien's discussion shows that distortions of voltage distribution are considerable in the attempts to clamp i_{NA} but much less pronounced in the case of a small, slowly changing outward current like i_{K2} where the degree of non-uniformity should be small (Tsien, 1974a; Jack et al. 1975).

Results

Local Anaesthetics do not Cause any Shift of s_∞ in the Presence of Adrenaline

Weld and Bigger (1976) showed that lidocaine itself (1 mg/l) had no influences on the i_{K2} current system. After the administration of adrenaline and shifting the *s*-kinetics in the depolarizing direction (5 preparations), procaine ($7.3 \cdot 10^{-4}$ M) was not able to restore the kinetics towards their original position (Fig. 1, 1 preparation). It is shown in Figs. 2 and 3 that the additional application of a beta-adrenoceptor blocker (atenolol $38 \cdot 10^{-6}$ M) is needed to cause a backshift of the *s*-kinetics (3 preparations). In Fig. 1, panel A shows the tracing of the clamp current under control conditions: The most important and characteristic feature is that the current tails on the return to the holding potential (i_B, see also inset in Fig. 2A) of −80 mV following depolarizing and hyperpolarizing clamp pulses are almost equal in their amplitudes. Panel B shows the wellknown alteration measured 30 min after the application of adrenaline ($5.5 \cdot 10^{-6}$ M) (Hauswirth et al., 1968): The current tail on the return to the holding potential following depolarization to −50 mV is increased in its amplitude and somewhat accelerated; following the hyperpolarizing clamp to −90 mV the current record shows virtually no remaining time dependence. In addition, the current level at the holding potential is shifted in the inward direction as described previously by Hauswirth et al. (1968) and indicated in Fig. 2 by arrows. Panel C demonstrates that, in the presence of adrenaline, there is essentially no change 30 min after the addition of procaine ($7.3 \cdot 10^{-4}$ M) (1 experiment). Although the time constants of the currents during and following depolarization to −50 mV are further accelerated, this has to be regarded as the full development of the adrenaline effect and was seen already prior to the administration of procaine. Finally, in the last panel it is shown that the beta-adrenoceptor blocking agent atenolol ($38 \cdot 10^{-6}$ M) cause a backshift of the voltage dependence of the *s*-kinetics in the presence of adrenaline and procaine (3 experiments): the time courses of the time dependent currents during and following the depolarizing clamp are slower again and the amplitude of the current tail of the return to the holding potential is smaller. Moreover, the occurrence of time dependent currents in response to hyperpolarization can be observed. Figure 2A shows

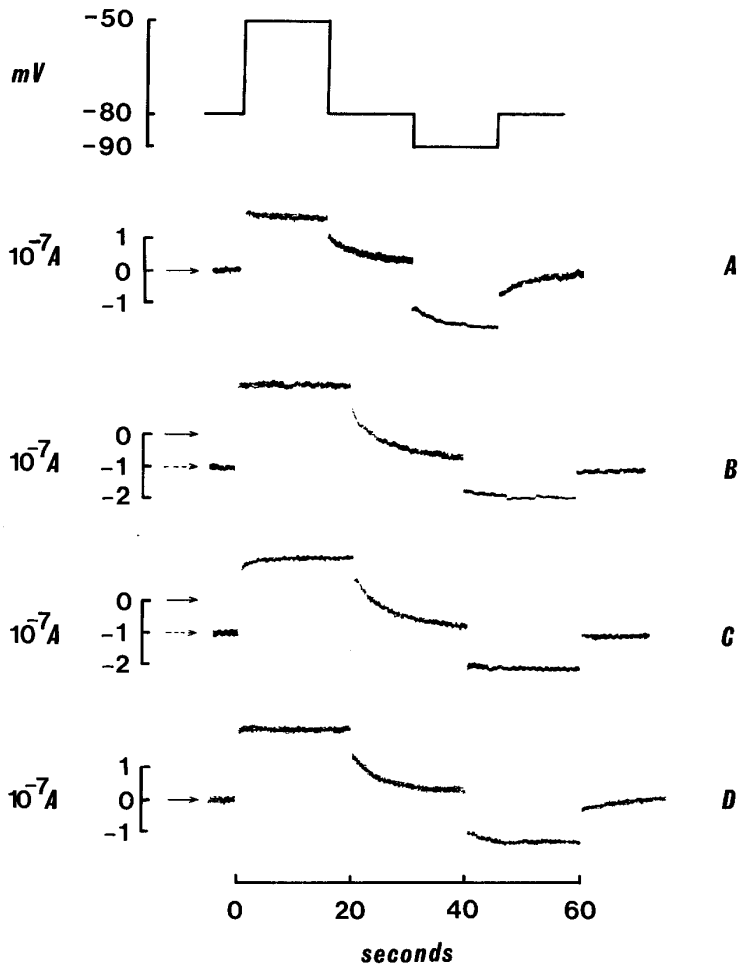

Fig. 1
One example of original records of membrane currents of a Purkinje fibre as responses to long lasting depolarizations and hyperpolarizations under various conditions. Holding potential: −80 mV. *Panel A:* Control; *Panel B:* Influence of adrenaline ($5.5 \cdot 10^{-6}$ M); *Panel C:* Effect of procaine ($730 \cdot 10^{-6}$ M) plus adrenaline. Note that this tracing is practically identical with that in *Panel B*; *Panel D:* Effect of the beta-adrenoceptor blocker atenolol ($38 \cdot 10^{-6}$ M) in the presence of adrenaline ($5.5 \cdot 10^{-6}$ M) and procaine ($730 \cdot 10^{-6}$ M). The beta blocker diminishes the amplitude of the tail current following depolarization and restores the time dependent of the current following hyperpolarization. Ordinate: top: Membrane voltage; below (*Panel A–D*): membrane current. Abscissa: time; Starting the experiment, the pulses as well as the intervalls lasted for 15 s. With adrenaline, the time constant at the depolarized voltage level was slowed and the amplitude of the current on the return to the holding potentials was increased. Therefore, *Panel B*, *C* and *D* show durations of pulses as well as of the intervalls of 20 s. Horizontal arrows → indicate control holding current; ⇢ indicate holding current shifted in inward direction in the presence of adrenaline and procaine

the curve relating the steady state degree of activation of i_{K2} and the membrane potential measured as the current tails (i_B) on the return to the holding potential under the conditions mentioned above.

Adrenaline shifts s_∞ (the normalized curve of Fig. 2A) in the depolarizing direction (Hauswirth et al., 1968; Tsien, 1974a) (Fig. 2B). The current tails obtained with procaine in the presence of adrenaline do not show any additional shift of the curve. The beta-adrenoceptor blocking agent, however, produces a substantial backshift although not entirely to the original position. The average shift of the *s*-kinetics caused by adrenaline ($5.5 \cdot 10^{-6}$ M) amounted to 20 mV ($\pm$ 2.2 mV); the backshift ranged close to 2/3 of the adrenaline shift with atenolol ($38 \cdot 10^{-6}$ M) or (–)-penbutolol ($17 \cdot 10^{-6}$ M) (Fig. 6) independently of the degree of the shift caused by a particular concentration of adrenaline ($5.5 \cdot 10^{-6}$ M). With atenolol (racemate) as well as with (+)- or (–)-penbutolol (Fig. 6) the backshift of the *s*-kinetics was not complete. On the other hand, Hauswirth et al. (1968) obtained a complete remission (see also Noble 1975) with a comparable concentration of a beta-adrenoceptor blocker (pronethalol $4 \cdot 10^{-6}$ M). However, the results of Hauswirth et al. (1968) were presumably due to the lower adrenaline concentration ($2.5 \cdot 10^{-6}$ M). Nevertheless, it is shown in Fig. 6 that the effect of the beta-adrenoceptor blocker is dependent upon the concentration administered and whether the (+)- or (–)-isomere is applied. This is shown in Fig. 2B more clearly: The normalized s_∞ curve is shifted by adrenaline by 18.5 mV in the positive direction; with the additional application of procaine no further shift occurs. The betablocker causes a shift by 12 mV in the negative direction which

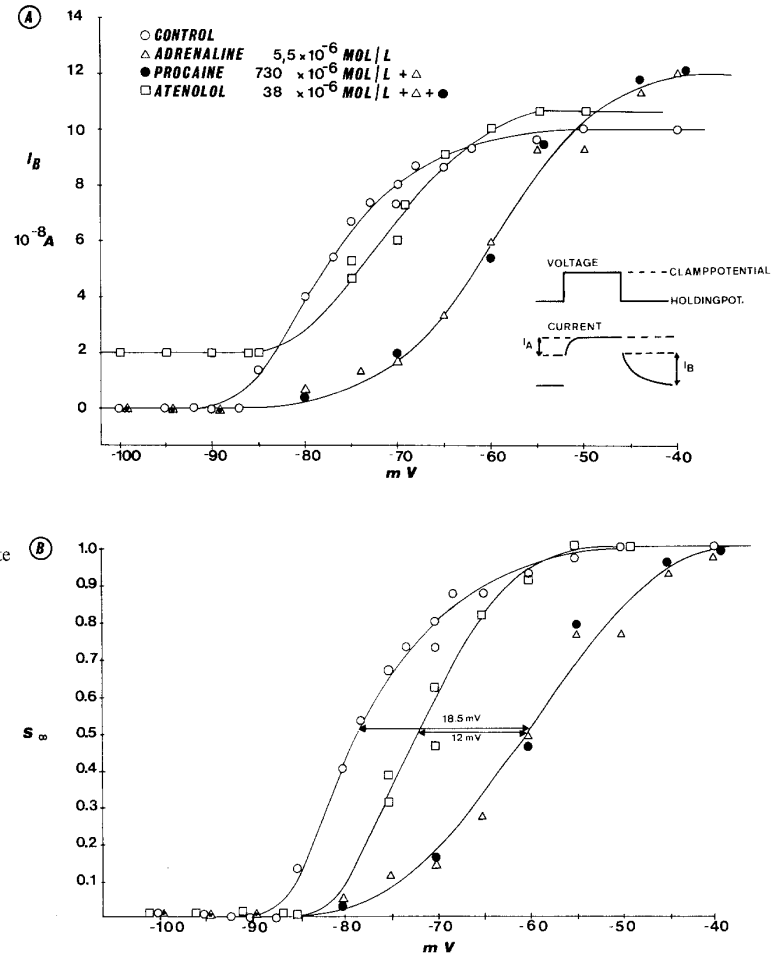

Fig. 2
(A) Steady state degree of activation of i_{K2} measured as the amplitudes of current tails on return to the holding potential under various conditions (I_B – see inset). (○) control; (△) adrenaline ($5.5 \cdot 10^{-6}$ M); (●) procaine ($730 \cdot 10^{-6}$ M) plus adrenaline; (□) atenolol ($38 \cdot 10^{-6}$ M) plus adrenaline and procaine. Note that procaine does not cause any further shift of the activation curve in the presence of adrenaline. The voltage dependence of the steady state degree of activation is again shifted in the negative direction by the additional application of the beta-blocker. Ordinate: current in 10^{-8} Ampere; Abscissa: membrane voltage in mVolt. **(B)** Normalized curves (s_∞) Here the voltage shift of the curves can be observed as 18.5 mV in the depolarizing direction by adrenaline and 12 mV in the backward direction by the beta-blocker. Ordinate: arbitrary units for the activation degree between 0 and 1 for s_∞. Abscissa: membrane voltage in mVolt

means that the catecholamine effect is compensated by two thirds using this particular concentration ($38 \cdot 10^{-6}$ M) of the beta-adrenoceptor blocking agent.

According to the Hodgkin-Huxley theory the time constants showed a behaviour similar to that of the activation curves. Figure 3A shows the amplitudes of current tails on the return to the holding potential following voltage clamp pulses of different duration to −40 mV and −50 mV. At both potentials, this was done under control conditions, adrenaline and the beta-blocker. It is shown that at both voltage levels the time constant is slowed under the influence of adrenaline and again accelerated in the presence of adrenaline and the betablocker.

The effects of adrenaline and the beta-adrenoceptor blocking agent on the rate of change of i_{K2} is summarized in Fig. 3B showing the reciprocals of the time constant in relation to the membrane potential. As already known from Figs. 2B and 3A, the *s*-kinetics are shifted in the depolarizing direction by adrenaline and restored to a large extent by the betablocker.

The Shift of the Rate Constants

According to the Hodgkin-Huxley theory, when $ds/dt = O$, the steady state value of s follows as:

$$s_\infty = \frac{\alpha_s}{a_s + \beta_s} \tag{3}$$

and

$$\tau^{-1} = \alpha_s + \beta_s. \tag{4}$$

α_s and β_s are the rate constants of the foreward and backward reactions (see page 9) which are voltage

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.