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CARDIAC PACEMAKER OSCILLATION AND ITS MODULATION BY AUTONOMIC TRANSMITTERS

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SUMMARY

1. A scheme is presented which summarizes the activation and de-activation of the membrane currents which underlie pacemaking in the natural pacemaker of the heart.

2. Experimental evidence (mostly obtained using the voltage-clamp technique) for the properties of the time-dependent membrane currents in pacemaking tissue of the frog and the rabbit is discussed.

3. The mode of the inhibitory action of acetylcholine on pacemaker cells is considered. In the amphibian pacemaker cell, acetylcholine probably reduces slow inward current (as it certainly does in amphibian atrium) but in mammalian sino-atrial node it seems that such action, if present at all, is much less marked. In the pacemakers of both amphibian and mammal, acetylcholine greatly increases outward potassium current and there is recent evidence that it may do so by opening up a special acetylcholine-activated potassium channel.

4. Adrenaline greatly increases the slow inward current in pacemaker as in other cardiac tissues. This increase, together with (in mammal at least) an increased change of an additional pacemaking current, overrides an adrenaline-induced increase in outward current and leads to acceleration of the pacemaking rate.

5. The Appendix contains a brief consideration of the experimental and theoretical basis for the method of exponential separation of outward current components in the presence of the extracellular potassium accumulation that inevitably accompanies the flow of outward membrane current.

INTRODUCTION

The heart, unlike most other biological oscillators, is a familiar part of human experience. Familiar too are the modulations in frequency: the heart that 'stands still with fear' or, like Macbeth's, 'knocks at the ribs'. Physiologists have known for some time that such changes are brought about by the autonomic transmitters acetylcholine and adrenaline, but how these substances act on the pacemaker at a cellular level is still being actively investigated.

Over the past two decades intracellular recording and voltage-clamp techniques have led to great advances in the understanding of the cellular events in many parts of the

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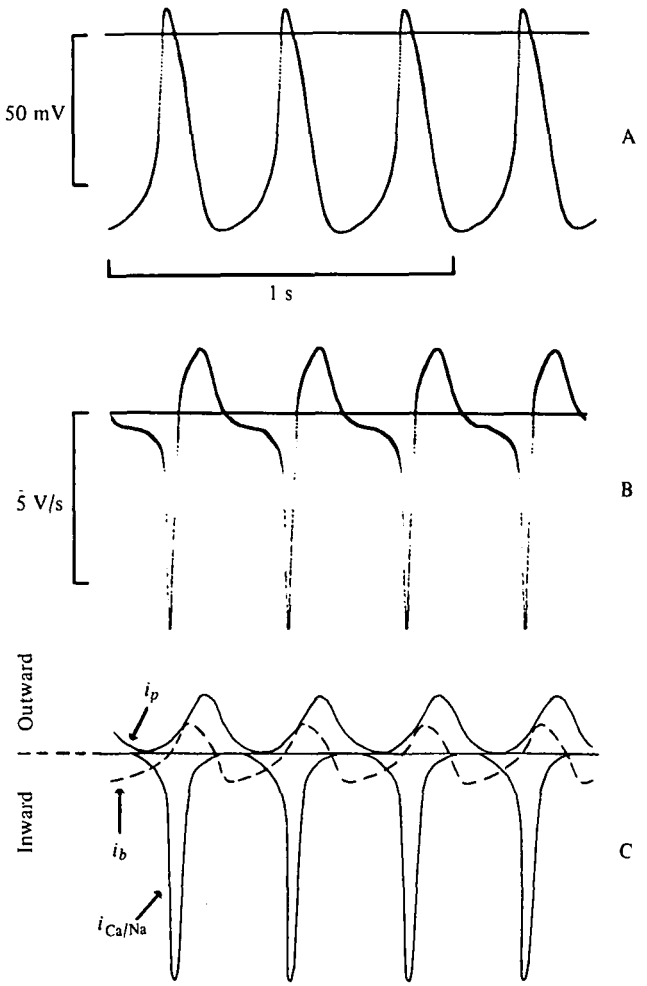

Fig. 1. (A) Transmembrane voltage record recorded with a microelectrode from a spontaneously pacing preparation of rabbit sino-atrial node. (B) Simultaneous record of rate of change of voltage (proportional to total membrane current). Horizontal line, zero level. (C) Diagram of the currents known to be involved in pacemaker activity. The horizontal line represents zero current level. i_p, 'Pacemaker' or time-dependent outward current. $i_{Ca/Na}$, Slow inward time-dependent current. i_b, Probable time course of total 'background' or time-independent current. (This comprises an inward background current carried probably by sodium ions and outward background current (i_{K_1}) carried by potassium ions.)

heart. As William Harvey himself noted, nearly all parts of the heart when separated from each other can develop spontaneous beating (Harvey, 1628). Recently, however, it has become clear that the membrane currents underlying pacemaking are not the same in all regions of the heart and one cannot extrapolate directly from, for example, the mechanism of pacemaking in the Purkinje cell to that of the natural pacemaker. There are considerable technical difficulties in applying the voltage clamp to the relatively small cells of the sinus region so that it is only in the past few years that direct evidence has been obtained of the nature of the oscillatory activity of the natural pacemaker.

A detailed review of pacemaker mechanisms has recently appeared (Irisawa, 1978). Rather than cover the same ground again, we shall largely confine ourselves to describing in context work on natural pacemakers with which we have been directly associated: voltage clamp of the sinus venosus of the frog using the double sucrose-gap technique and of the sino-atrial node of the rabbit using two microelectrodes.

Fig. 1 shows a simple scheme for the system of membrane currents underlying pacemaking. It was originally based on experimental data from voltage-clamp experiments on tissue from the sinus venosus region of the heart of *Rana catesbeiana* (see Brown, Giles & Noble, 1977*b*) but can also be applied with little alteration to mammalian SA node.

Four action potentials and the pacemaker depolarizations between them are shown at the top of the figure. The rate of change of voltage, dV/dt (proportional to the total membrane current), is shown in Fig. 1 B. In Fig. 1 C the direction and time course of the various membrane currents are indicated. Outward current, i_p, is switched on at the relatively positive membrane potentials reached during the action potential. It assists in repolarizing the membrane and then decays at the more negative potentials of the diastolic interval. This declining outward current coupled with the time-independent background current (i_b, dotted line Fig. 1 C) gives a resultant net inward current which depolarizes the membrane fairly slowly: the pacemaker depolarization. During the later part of the pacemaker depolarization, the threshold of the time-dependent inward current (i_{Ca^{2+}/Na^+}, Fig. 1 C) is reached giving more rapid depolarization which leads to the action potential upstroke. $I_{Ca/Na}$ switches off fairly rapidly at the more positive potentials of the action potential peak, and this contributes to the repolarization of the membrane.

A system of membrane currents of this kind would certainly lead to pacemaking, and although it may be oversimplified and there are still some remaining uncertainties about the characteristics of some of the membrane currents involved, we believe the scheme to be broadly correct in sinus cells. We shall outline some of the experimental evidence on which our knowledge of the membrane currents in frog sinus is based before indicating some possible differences found in the mammalian sinus node.

The time-dependent inward current (i_{Ca^{2+}/Na^+}) in frog sinus venosus

In Fig. 2 are shown the electrical responses of an isolated pacemaker preparation of the frog *Rana catesbeiana* recorded across a sucrose gap. In Fig. 2 A the spontaneous electrical activity of this preparation is shown before (leading trace) and after (following trace) the application of tetrodotoxin (TTX) in a concentration of $2{\cdot}0 \times 10^{-6}$ g/ml. It can be seen that this substance, which blocks the fast inward sodium channel in nerve and muscle and in other regions of the heart, has little effect on the overall voltage activity of this preparation nor on the rate of rise of the action potential (Fig. 2 B). Not all sinus preparations in the frog are insensitive to TTX. Fig. 2 C shows a 'follower' type of preparation, in which the regenerative activity was blocked by TTX. However, the fact that, when TTX is applied to the sinus venosus and atria of a frog which have been opened up in a dissecting dish, most of the sinus tissue continues beating as before while the atria become quiescent, indicates that in the majority of cells of the pacemaker region the membrane channel underlying the upstroke of the action potential is not the fast sodium channel. In voltage-clamp

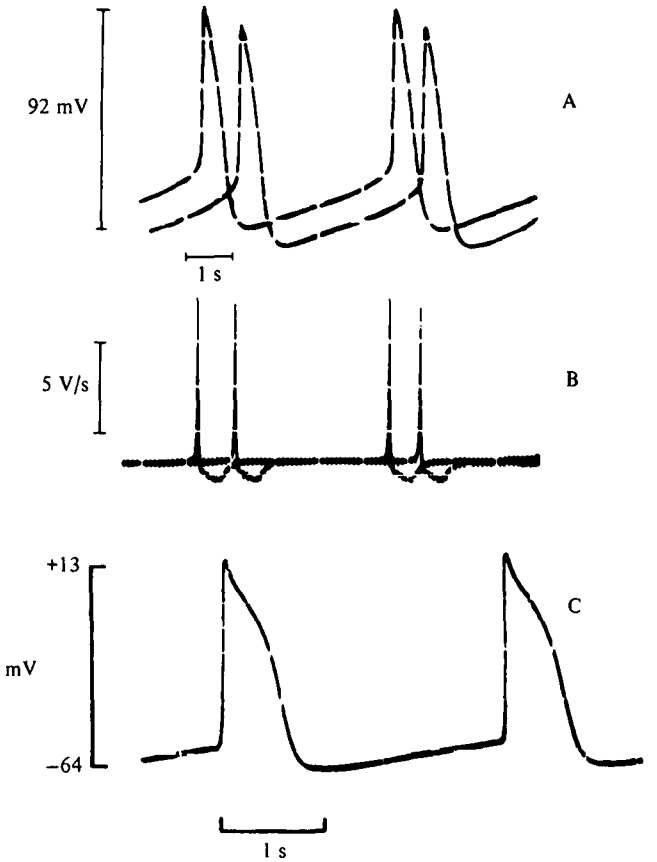

Fig. 2. (A) Electrical responses recorded across a sucrose gap from an isolated pacemaker preparation of *Rana catesbeiana* before (leading trace) and after (following trace) application of tetrodotoxin. Although it did bring about some hyperpolarization, TTX caused little change in the rate of rise of the action potentials (B), indicating that the preparation showed primary pacemaker activity. (C) Spontaneous activity in a 'follower' sinus preparation from frog (which proved to be TTX-sensitive). The diastolic depolarization is shallower and there is a sharper transition to action potential upstroke than in primary pacemaker tissue (from Brown *et al.* 1977*b*).

experiments we have been able to show that the inward time-dependent depolarizing current in frog sinus is abolished by manganese (Brown *et al.* 1977*b*), indicating that it is the slow inward (Ca^{2+}/Na^{+}) current which has been described in many other regions of the heart, in most of which it is present together with the fast sodium system (Rougier *et al.* 1969; Beeler & Reuter, 1970; Reuter, 1973).

Is there any obvious reason why the cells of the pacemaker should for the most part lack the fast sodium system and have only the slow inward system, resulting in a much more slowly rising action potential (in frog 5–10 V/s as against about 100 V/s)? The significance of the lower rate of spread within the sinus region and of such relatively slowly rising action potentials in either the prevention or genesis of arrhythmias is not yet clear. However, the lack of a fast inward current, and the resulting low speed of action potential propagation, might, under normal conditions, help prevent re-entrant tachycardia.

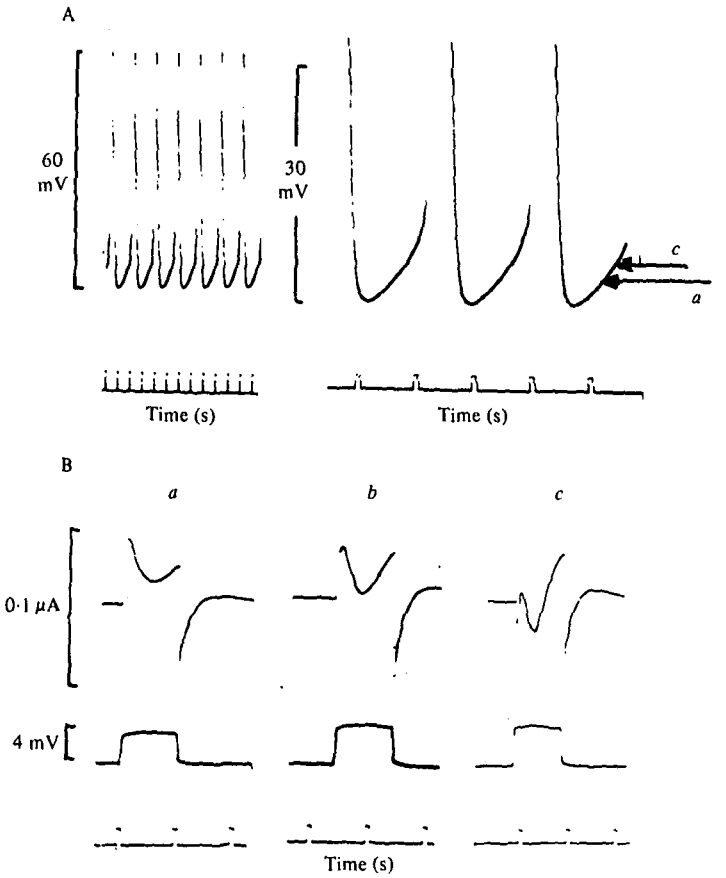

Fig. 3. (A) Spontaneous activity of a frog sinus preparation in TTX-Ringer solution recorded across a sucrose gap. Left, lower gain record. Right, higher gain record to show the pacemaker depolarization in more detail. Levels to which voltage-clamp pulses *a* and *c* below depolarized the membrane are marked against the pacemaker depolarization. (B) The preparation was then voltage clamped at the maximum diastolic potential and depolarizing pulses of +4, +5 and +6 mV applied (*a*, *b* and *c*). The corresponding current records are shown above each voltage-clamp pulse and illustrate the onset of the slow inward ($i_{Ca/Na}$) current (from Brown *et al.* 1977*b*).

The level of membrane potential at which the slow inward current ($i_{Ca^{2+}/Na^{+}}$) is activated is indicated in the experiment shown in Fig. 3. Here the currents in response to very small voltage-clamp depolarizations into the pacemaker range were recorded, and it can be seen (Fig. 3B) that a potential and time-dependent inward current is activated by pulses that displace the potential into the latter part of the pacemaker potential range. An additional time-dependent current (which we shall call i_f) can be demonstrated in most frog sinus preparations but since it is unmasked only by hyperpolarizing the preparations negative to the maximum diastolic potential (see Fig. 4) it seems uncertain whether, in the frog, it can play any part in pacemaking. A similar current is seen in the mammalian sinus (see below).

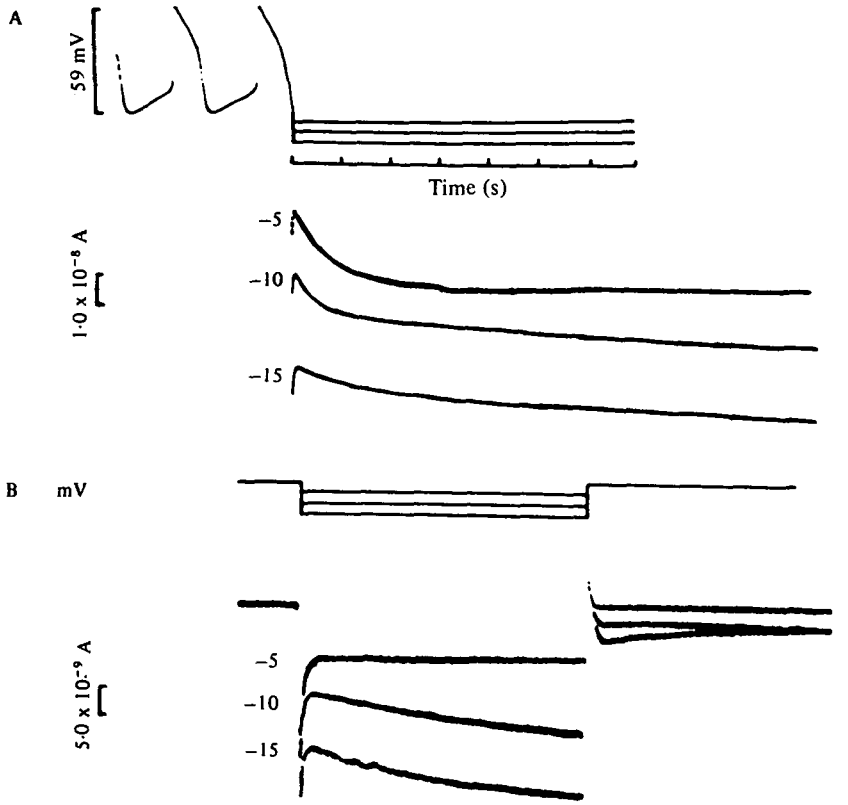

Fig. 4. Evidence for the presence of an additional current (i_f) in frog sinus (double sucrose-gap technique). (A) Voltage clamp applied directly to a spontaneously active preparation at 5, 10 and 15 mV negative to its maximum diastolic potential. Voltage records above, corresponding current records below. At 5 mV negative to the maximum diastolic potential, apparent deactivation of an outward current occurred and the trace then remained flat. At 10 and 15 mV negative to the maximum diastolic potential, the current continued to drift downwards throughout the hyperpolarizing clamps, indicating activation of another current.

(B) Top: voltage protocol; the same preparation was clamped at the maximum diastolic potential and hyperpolarizing clamps given. Below: the current records in response to 10 and 15 mV hyperpolarizations confirmed the activation of the additional current, i_f (from Brown *et al.* 1977*b*).

Time-dependent outward current in frog sinus venosus

When the voltage clamp is switched on during a pacemaker potential to a level corresponding to that of the maximum diastolic potential of the preparation, a relatively large current which decays slowly with time is recorded (Fig. 5). Since the magnitude of the current recorded at the maximum diastolic potential (Fig. 5, point 1) is greater than that recorded midway through the pacemaker depolarization (Fig. 5, point 2) we can conclude that the current change recorded during voltage clamping is the current change which occurs naturally during the pacemaker depolarization. However, from this experiment we cannot tell whether this pacemaker current is an outward or an inward current. To determine this we must find its reversal potential.

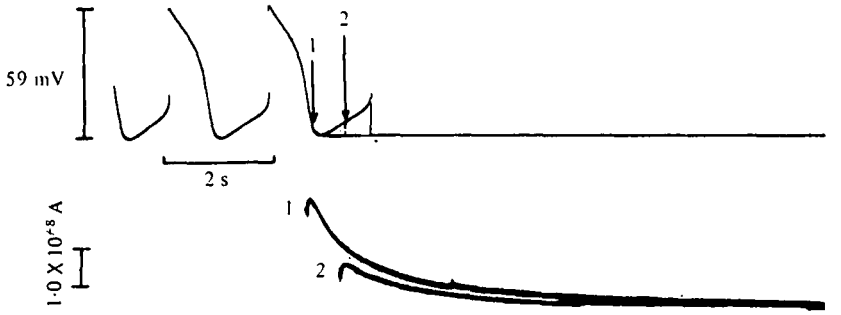

Fig. 5. Frog sinus preparation; double sucrose gap technique. Current recorded (bottom traces) when the voltage clamp was switched on at two different times, 1 and 2, during a spontaneous pacemaker depolarization (from Brown *et al.* 1977*b*).

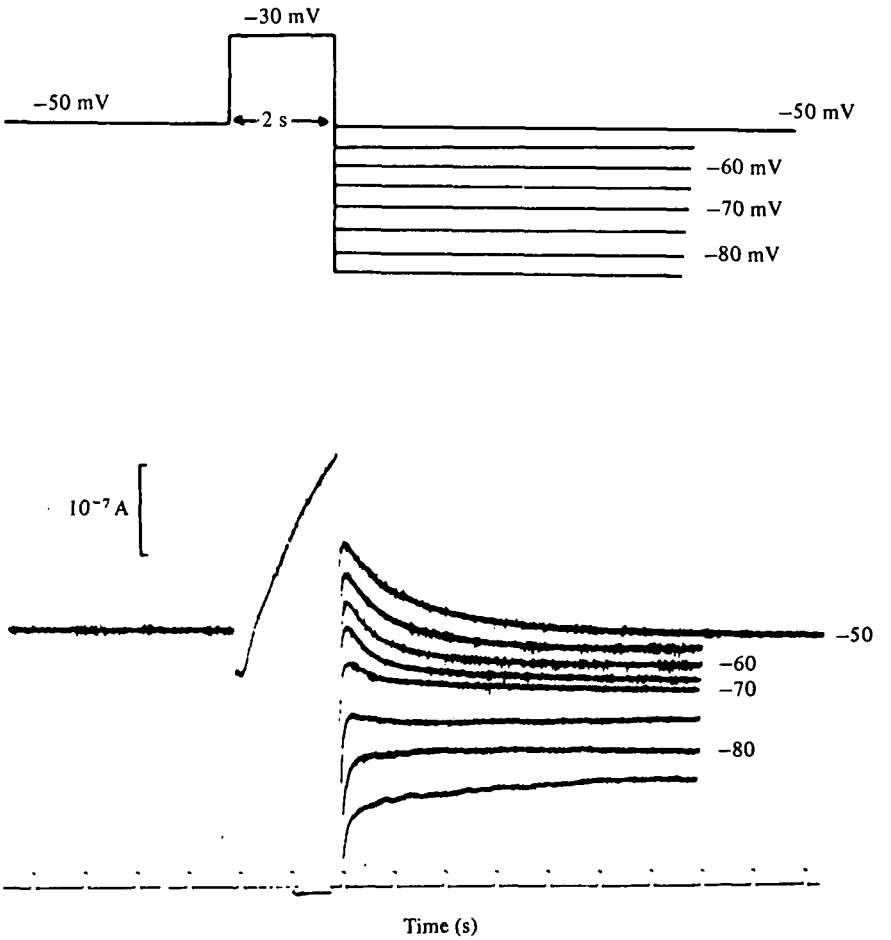

Fig. 6. Frog sinus preparation; double sucrose gap technique. Voltage-clamp protocol (above) and simultaneous current records (below). After a constant 2·0 s small depolarizing pulse from −50 mV to −30 mV, a 10 s hyperpolarizing clamp pulse was applied to the potential levels indicated. Note current reversal near −80 mV (from Brown *et al.* 1977*b*).

Fig. 6 illustrates that this 'pacemaker' current (i_p) contains an outward component with a reversal potential near −80 mV. In order to activate this component of outward current fairly small depolarizing voltage-clamp pulses (+20 mV) were applied to a preparation for 2 s from a holding potential of −50 mV. After each pulse the membrane was returned to progressively more hyperpolarized levels. During the pulse, after the relatively rapid onset and inactivation of the slow inward current, there was an onset of time-dependent outward current. The decay of current after the pulse reverses direction when the membrane is clamped back to potentials more negative than −80 mV. (It should be noted that results of the kind shown in Fig. 6 are very often masked by the slow time-dependent current (i_f) shown in Fig. 4.) Since potassium-sensitive microelectrodes have indicated that the potassium equilibrium potential in frog sinus is close to −88 mV (Walker & Ladle, 1973) a simple interpretation of the experiment shown in Fig. 6 is that the decay of a current carried by potassium ions and activated during the action potential controls the rate of development of the pacemaker depolarization in frog heart. However, when larger voltage-clamp depolarizations than those shown in the experiment in Fig. 6 are given to a preparation, analysis of the current 'tails' after the pulses shows that there are two components of time-dependent outward current in this tissue and also gives information about the voltage dependence and kinetics of these two components. This analysis relies upon exponential separation of components of outward current. In the Appendix to this article will be found a discussion of the experimental and theoretical evidence justifying this method of separating components of outward current in the presence of the accumulation of potassium ions which inevitably accompanies them, together with a consideration of the accuracy such analysis can achieve.

The voltage-dependent and kinetic behaviour of the outward currents in frog sinus

Fig. 7 shows normalized (i.e. plotted on a scale from 0 to 1) activation curves for the two time-dependent outward currents obtained by semi-logarithmic analysis (see Appendix) in a frog sinus preparation. The filled circles give the activation curve for the more slowly decaying component of time-dependent outward current, i_{slow}, and it can be seen that it lies further negative on the voltage axis than the activation curve for the more rapidly deactivating component, i_{fast} (crosses). (It is evident from the position of these activation curves why the pulses given in the experiment shown in Fig. 6, from −50 mV to −30 mV, activated predominantly one component, i_{slow}.) The most important result to notice from these activation curves is that both components are fully activated at zero mV. This would be expected in a preparation which is never under normal circumstances depolarized to more than about +10 mV, and contrasts strongly with the situation found in frog *atrium*, where at zero mV two similar components of outward current are only 55% and 40% activated (Brown, Giles & Noble, 1977*b*). Do both these components of sinus outward current function in repolarization and pacemaker activity? In Fig. 8 the amounts of activation of the two components of time-dependent outward current in sinus are plotted as a function of the duration of the preceding depolarization. Square voltage-clamp pulses from a holding potential of −50 to +10 mV for varying times were used, and reasonably large positive current tails were recorded after even very short depolarizations. The

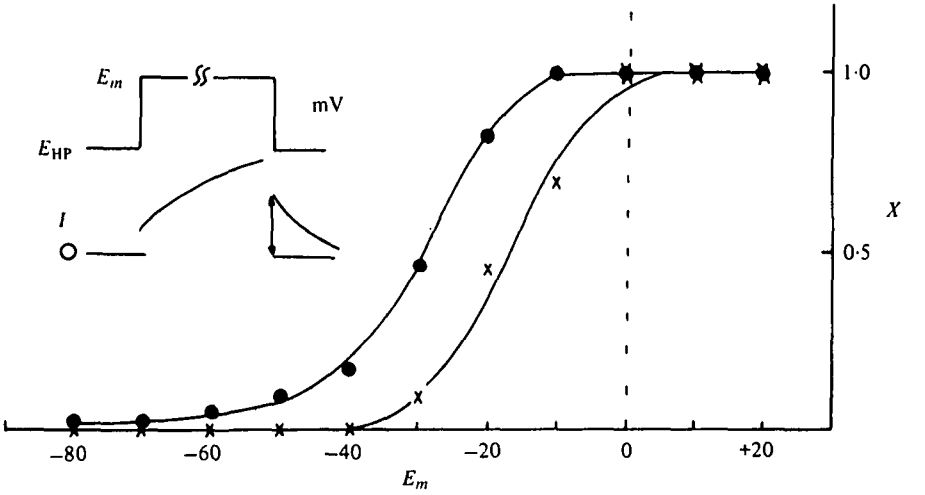

Fig. 7. Activation curves for the two components of time-dependent outward current in frog sinus. ●, more slowly decaying component (i_{slow}); crosses, more rapidly decaying component (i_{fast}). The inset indicates how the current tail was obtained; it was then plotted semi-logarithmically and the two components were separated as described in the Appendix (in association with Fig. 19). (from Brown *et al.* 1977*b*).

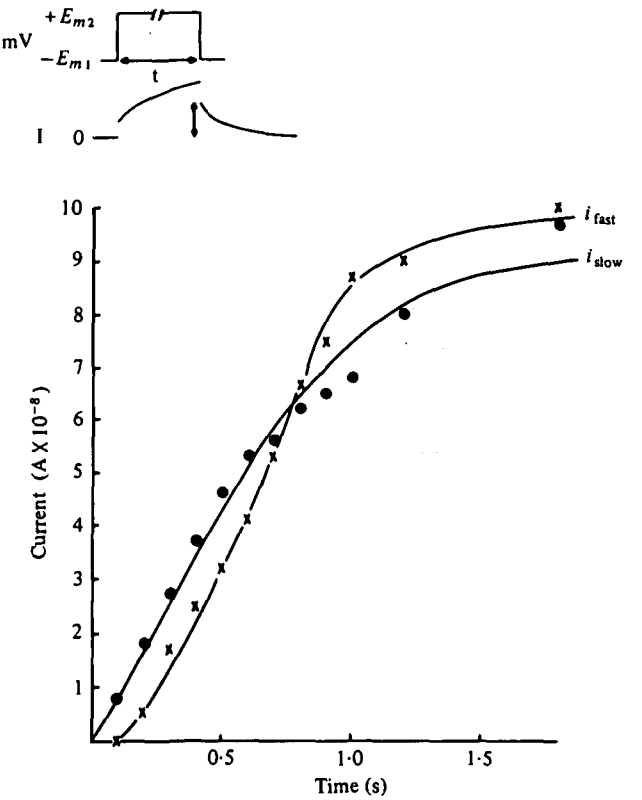

Fig. 8. The amount of activation of the two components of time-dependent outward current in frog sinus as a function of the duration of the preceding pulse. The inset indicates the method used to obtain the current tail ($-E_m = -50$ mV; $+E_{m_2} = +10$ mV), which was again separated into exponentially decaying components as described in the Appendix (in association with Fig. 19). (from Brown *et al.* 1977*b*).

two outward current components were again separated by semi-logarithmic analysis (see Appendix). The filled circles show the amount of i_{slow} present and the crosses represent i_{fast}. It can be seen that i_{fast} is present in all current tails following depolarizations lasting longer than 100 ms. These results indicate that both outward currents may be involved in repolarization and in regulating sinus pacemaker activity.

To check this, current records obtained when the voltage clamp was applied directly to spontaneously active preparations were analysed carefully. When any of these records, obtained at different times during the pacemaker depolarization, was plotted semilogarithmically the presence of two components was apparent. Both of the time-dependent outward currents, i_{fast} and i_{slow}, appear to deactivate throughout the duration of the pacemaker depolarization, and furthermore they decay in the manner expected of independent components, i.e. the ratio of the amplitude of i_{fast} and i_{slow} decreases the longer the diastolic depolarization has continued before the clamp is applied. This result provides a marked contrast between sinus and induced atrial pacemaker activity where only one component, $i_{x,slow}$, is seen to underlie the pacemaker depolarization (Brown, Clark & Noble, 1972). However, it is unlikely to make a great difference when qualitatively formulating a possible sinus pacemaker model (see Fig. 1), although it would have to be taken into account for any quantitative computation.

Voltage-clamp experiments on the mammalian SA node; the two-microelectrode method

The double sucrose gap method which has proved so successful for voltage clamping amphibian sinus venosus is unsatisfactory for mammalian SA node. This could be due to the particular geometry of this tissue or to the fact that it contains a high proportion of connective tissue cells. Voltage clamping of mammalian sinus has been much more successful using two microelectrodes to clamp very small preparations (*ca.* 300 × 300 μm) that are obtained by cutting and ligaturing strips of tissue from the sino-atrial node of the rabbit (Noma & Irisawa, 1976).

Several different types of cell have been described in the mammalian SA node. We believe that the very small preparations we use are largely composed of true pacemaker cells (also known as nodal cells (Irisawa, 1978) or dominant pacemakers (Lipsius & Vassalle, 1978)) and we shall in this section consider evidence only from small preparations of this type where the possibility of electrotonic interaction from cells of other types (transitional cells or atrial cells) is minimal.

Fig. 9 shows the electrical changes recorded with a single microelectrode from such a spontaneously pacing, small preparation of rabbit sinus. A total potential swing of between 70 and 80 mV is commonly recorded from our preparations. We usually find a maximum diastolic potential of about -60 to -70 mV and the 'take off point' of the regenerative upstroke near -40 mV.

Inward currents in SA node

Pacemaker insensitivity to tetrodotoxin represents one of the methods used to distinguish true pacemaker cells from follower cells in sinus tissue (Yamagishi & Sano, 1966). It seems that in the mammalian SA node the TTX insensitivity of spontaneous pacemaking is due to the relatively positive range of potential at which such activity

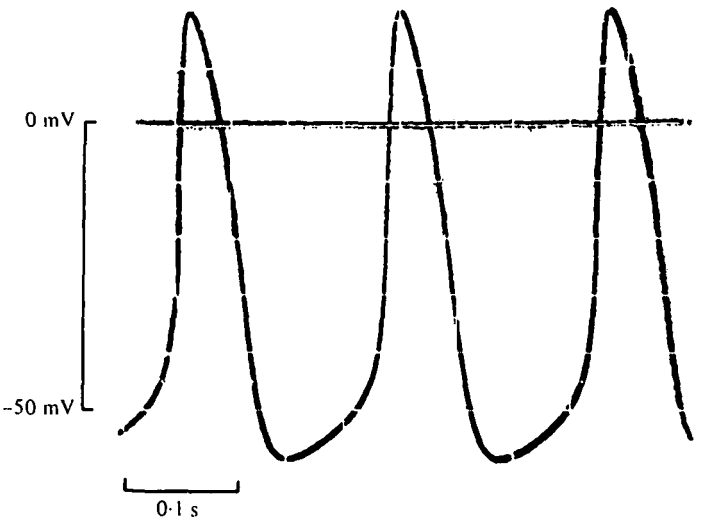

Fig. 9. Intracellular record of spontaneous activity in a small preparation (300 × 300 μm) of rabbit sino-atrial node.

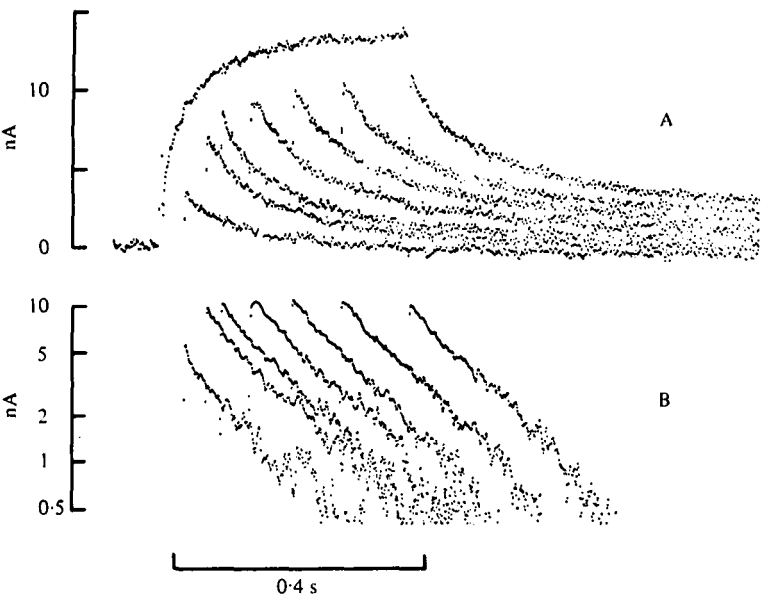

Fig. 10. (A) Time course of onset and decay of outward current in the rabbit sino-atrial node recorded during and after voltage-clamp depolarizations of various durations from a holding potential of −40 to −10 mV (envelope test). The curves are digitalized averages of 3–5 current traces. (B) The early part of the same current tails are plotted on a semi-logarithmic scale showing that they can be fitted by single exponentials with the same time constant. (DiFrancesco, Noma & Trautwein, 1979.)

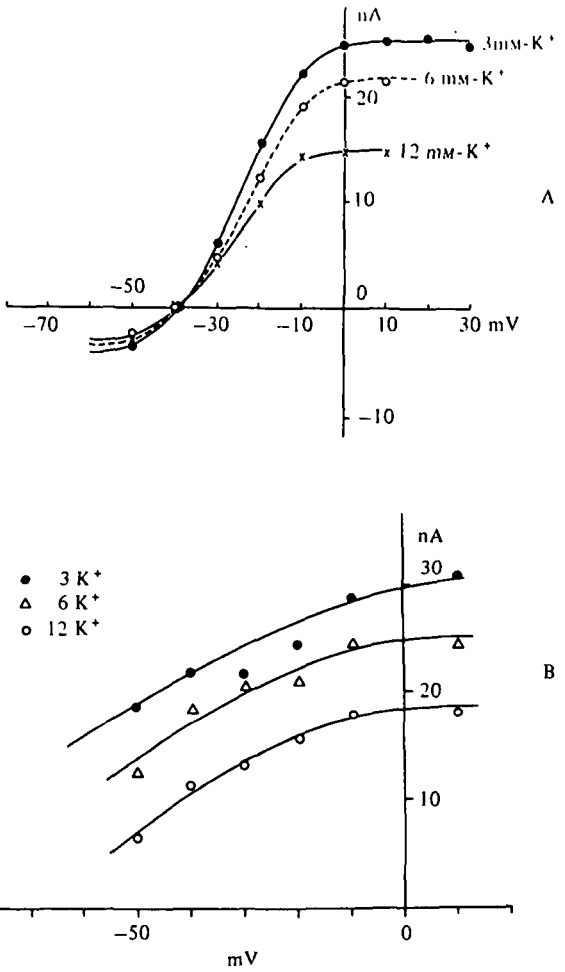

Fig. 11. (A) Activation curves for the outward, time-dependent current (i_p) in the SA node of the rabbit in different external potassium concentrations. Note that the activation curves decrease in amplitude in high potassium but if normalized can be fitted by the same theoretical curve. This implies that the potassium concentration does not affect the kinetics of i_p. (B) Fully activated I–V relationships of i_p in different potassium concentrations (same experiment as in (A)). No cross-over phenomenon is observed. (DiFrancesco, Noma & Trautwein, 1979.)

occurs, rather than to the absence of a TTX-sensitive channel. Thus Kreitner (1975) showed that if SA node is hyperpolarized by carbachol, a TTX-sensitive channel is revealed and Noma & Irisawa (1976*a*) have demonstrated that when the membrane is voltage-clamped negative to −40 to −50 mV a TTX-sensitive fast inward current is present at the onset of square depolarizing pulses in addition to the D600 and Mn^{2+}-sensitive slow inward current which is present alone when the membrane is clamped positive to −40 mV. At the normal 'take-off' potential of about −40 mV, the fast inward Na^+ channel is completely inactivated so that under normal conditions only slow inward (Ca^{2+}/Na^+) current underlies the action potential upstroke in true

pacemaker cells accounting for the slow rate of rise (10–20 V/s in rabbit SA node cells) and TTX-insensitivity.

The slow inward current is activated by depolarizations given from holding potentials in the range -50 to -20 mV and is primarily sensitive to Na^+ ions (Noma, Yanagihara & Irisawa, 1977), although changing the Ca^{2+} concentration also greatly affects the peak current recorded during a depolarizing clamp (Brown, DiFrancesco & Noble, unpublished). The time constant of deactivation of this current is about 30 ms at -20 mV (DiFrancesco & Noma, unpublished), which is comparable with the time taken by the action potential to repolarize the membrane. This current could, therefore, be important in determining action potential duration. Although it seems that this channel is analogous to the slow inward (Ca^{2+}/Na^+) channel in other regions of the heart (Rougier *et al.* 1969; Beeler & Reuter, 1970) a complete description of its kinetic properties in the sinus is not yet available. Both calcium and sodium ions evidently contribute to the slow inward current in the SA node although the extent to which each contributes is not entirely clear, and may not be the same as in other cardiac tissues (Reuter & Scholz, 1977*a*; Noma & Irisawa, 1974, 1976*c*). However, the blocking agents D600 and manganese ions appear to block the slow inward channel as effectively in SA node as they do in other cardiac tissues.

The changes the slow inward current undergoes under the influence of the autonomic transmitters are discussed below. (Fig. 15 shows voltage-clamp records of this current in relation to changes in adrenaline.)

Outward currents in the mammalian SA node

Noma & Irisawa (1976) have described a time-dependent outward current activated in the range -60 to $+10$ mV. They found that the reversal potential of this current depends on external potassium concentration in such a way as to suggest that it is mainly carried by potassium ions. More recent work (DiFrancesco, Noma & Trautwein, 1979) has shown that the time constants of onset and decay of i_p at the same potential are closely similar thus implying that the activation/deactivation of this current obey first-order kinetics. Fig. 10A shows a series of superimposed current traces obtained during and after application of $+20$ mV voltage-clamp pulses of various durations (envelope test). In Fig. 10B the tails are plotted on a semilogarithmic scale from which it is evident that the same single exponential time course is obtained for all pulse durations, at least during the first 500 ms of current decay.

It is interesting to note that, in contrast to that of the Purkinje fibre i_{K_1} current (Noble & Tsien, 1968) but like that of the Purkinje fibre i_x current (DiFrancesco & McNaughton, 1979), the fully activated current–voltage relation for the sinus outward current does not show 'cross-over' when the external potassium concentration, K_o, is altered. This can be seen in Fig. 11B, which shows the I/V relationships for the SA node time-dependent outward current in 3, 6 and 12 mM $[K^+]_o$. Fig. 11A also illustrates that altering the external potassium concentration does not shift the position of the activation curves for this current on the voltage axis, implying that the kinetics of this current are not changed by potassium.

When current tails lasting longer than 0·5–1·0 s are recorded after voltage-clamp depolarizations they contain an additional slow component. This component, if

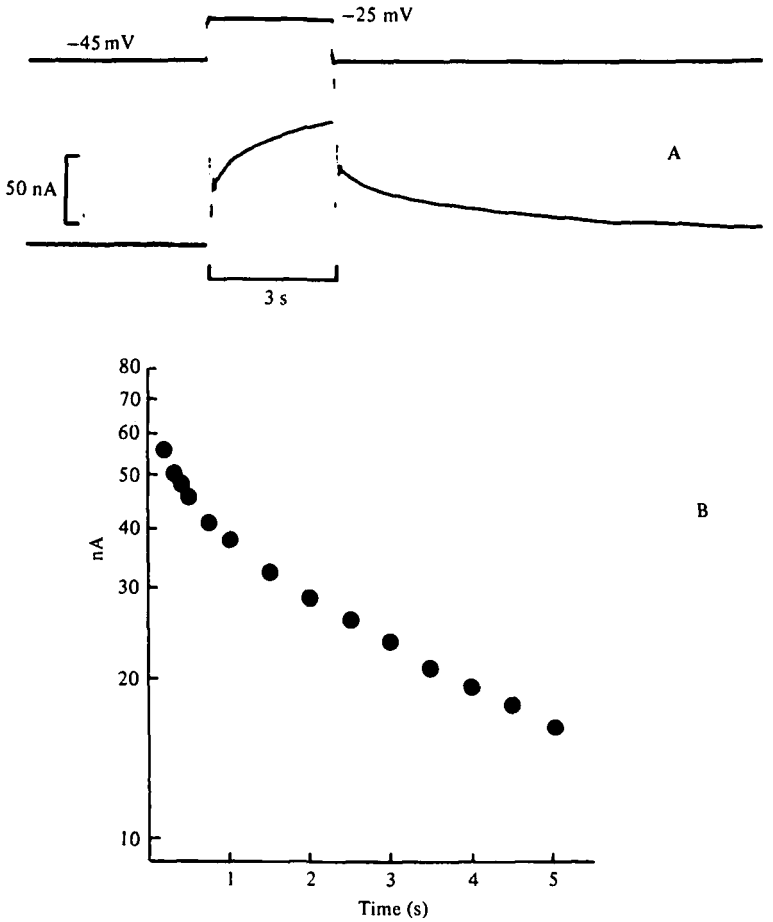

Fig. 12. Rabbit sino-atrial node: presence of a slow-component in the outward potassium current. (A) Current recorded during and after a 3 s voltage-clamp pulse from −45 to −25 mV. (B) Semilogarithmic plot of the current tail showing the presence of a slow component which can be shown to have a time constant of 5·5 s, while the remaining faster component has a time constant of 225 ms.

separated by exponential splitting methods (see Appendix) is seen to decay with a much slower time constant than does the fast component. Fig. 12 shows a record of such a multi-component decay tail recorded at −45 mV after a 3 s depolarization to −25 mV. Below is the semi-logarithmic plot of the same tail showing that it contains more than one exponentially decaying component. Here the time constant of decay for the fast component is 250 ms compared with 5·5 s for the slow component at −45 mV. It is possible that this slow component can be attributed to potassium ion accumulation.

A fourth time-dependent current in SA node

As in frog sinus venosus (see above, Fig. 4) another current is activated in the rabbit sinus at potentials negative to −50/−60 mV. (This current is shown in Fig. 16 below.) Little is known about this current but it has been shown that it disappears

when either Na^+ or Cl^- ions are removed from the external solution and for this reason it has been proposed that it is an inward current carried by one of these ions (Irisawa, 1978).

Recapitulation of the membrane currents involved in pacemaking

There is therefore considerable experimental evidence about the properties of the membrane currents underlying pacemaking in the natural pacemaker and those in the mammalian SA node are similar to those in frog sinus venosus: the diagram in Fig. 1 is a simple scheme of pacemaking, summarizing present ideas. In both frog and rabbit pacemakers it is primarily the TTX-resistant slow inward (Ca^{2+}/Na^+) current which underlies the action potential upstroke. Time-dependent outward current (comprising two separate components) is switched on during the action potential and is important in repolarization. At the more negative membrane potentials of the diastolic interval, this outward current decays fairly slowly, allowing the background (time-independent) current, which is at these potentials net inward, to depolarize the membrane: the pacemaker depolarization. When the membrane potential reaches the threshold of slow inward current activation, depolarization proceeds more rapidly and another action potential fires. In some, possibly in all, rabbit SA-node cells, an extra time-dependent current is activated within the pacemaker range and this may also be an important pacemaker current.

Inhibition of pacemaker activity by acetylcholine

Gaskell (1886) showed that stimulation of the vagus nerve caused a small hyperpolarization in tortoise auricle. More recently, microelectrode techniques have revealed marked hyperpolarization in sinus and atrial cells when the vagus is stimulated or acetylcholine is applied (Burgen & Terroux, 1953; Hutter & Trautwein, 1956, see Fig. 13 below) although in the atrium such hyperpolarization is less consistently present (Toda & West, 1967; Glitsch & Pott, 1978). Radioactive tracer studies suggest that the hyperpolarization is produced by an increase in the permeability of the membrane to potassium ions.

Acetylcholine action in the amphibian and reptile heart

In bullfrog atrium voltage-clamp studies using the double sucrose-gap technique have shown that acetylcholine blocks the slow inward (Ca^{2+}/Na^+) current (Giles, 1974; Giles & Tsien, 1975). Doses of acetylcholine between 3 and 5×10^{-8} M reduce the magnitude of the slow inward current by 50% without altering either the resting potential or the magnitude of the instantaneous background (potassium) current (Ikemoto & Goto, 1975; Giles & Noble, 1975). It has been further shown that reduction of slow inward (Ca^{2+}/Na^+) current and of the associated contractile response (again in bullfrog atrium) starts at doses of carbamylcholine as low as 10^{-8} M ($K_m = 10^{-7}$ M) whereas increase in potassium current starts only at 5×10^{-8} M and has a K_m of about 10^{-6} M (Garnier *et al.* 1978).

Thus in amphibian atrium reduction of the slow inward (Ca^{2+}/Na^+) current is an important action of acetylcholine. Is this the case in the sinus also?

Full voltage-clamp control of inward current in frog sinus trabeculae is hard to obtain. It is easier to record the maximum rate of rise (dV/dt) of the sinus action

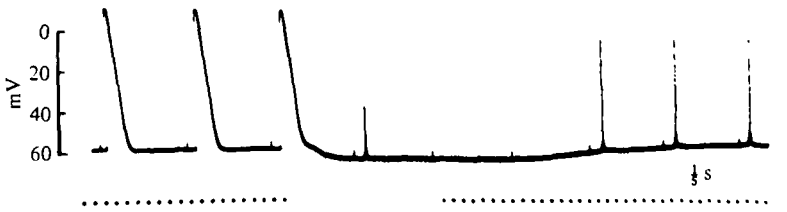

Fig. 13. Action and resting potentials in tortoise sinus venosus during and following stimulation of the vagus nerve, indicated by interruption in dotted line. During stimulation the membrane hyperpolarizes and action potentials are either very short (1st response) or entirely suppressed (second and third responses). After stimulation has ceased, the resting potential returns to its original level but the action potentials remain very short (4th, 5th and 6th responses). (Hutter & Trautwein, 1956.)

potential, and if this is done in the presence of tetrodotoxin it should give an indication of the amplitude of Ca^{2+}/Na^{+} current. Carbachol at a concentration of 5×10^{-7} M reduces the rate of rise of the sinus action potential without causing any hyperpolarization and the concentration has to be increased to 10^{-6} M before any significant hyperpolarization occurs (Brown, Giles & Noble, 1977*a*). This suggests that cholinomimetic substances do block slow inward current in amphibian sinus venosus at doses too low to increase potassium current. The method seems to be reliable, for adrenaline gives the opposite effect on action potential height and dV/dt (Brown, Giles & Noble, 1978), and it has also been shown using the voltage clamp technique that adrenaline increases the magnitude of the slow inward current both in the tortoise sinus venosus (Brown, Noble & Noble, 1978) and in the rabbit SA node (see below).

The hyperpolarizing effect of acetylcholine on the sinus venosus of amphibians and reptiles is well documented and $^{42}K^{+}$ efflux from tortoise sinus venosus is about 100 times greater for a given dose of acetylcholine than that from tortoise atrium (Giles, personal communication). It seems then that in the amphibian and reptile sinus venosus the actions of acetylcholine both on outward potassium current and on slow inward (Ca^{2+}/Na^{+}) current are important. This is indeed evident from some of the records of vagal action. Thus, the record shown in Fig. 13 (from Hutter & Trautwein, 1956), in addition to showing that vagal stimulation produces hyperpolarization in tortoise sinus venosus also shows that after the resting potential (and therefore the potassium conductance) have returned to normal, the action potentials are still short and spike-like, indicating a reduction of the Ca^{2+}/Na^{+} current.

Acetylcholine action in the mammalian heart

A suggestion that mammalian heart tissue may respond to acetylcholine rather differently from that of amphibians and reptiles comes from the finding of Ten Eick *et al.* (1976) (using the voltage clamp) that very large doses of acetylcholine ($5{\cdot}1 \times 10^{-6}$ M) are needed to block the slow inward (Ca^{2+}/Na^{+}) current in rabbit atrium.

Recording with a single microelectrode from the rabbit SA node, Kreitner (1975) found that the 2–8 mV hyperpolarization produced by $0{\cdot}5$ to 5×10^{-8} M carbamylcholine in rabbit SA node is accompanied by an increase in the maximum rate of rise of the action potential. The increase in dV/dt is abolished by tetrodotoxin, suggesting that it is due to activation of the fast inward sodium channel at more hyperpolarized potentials.

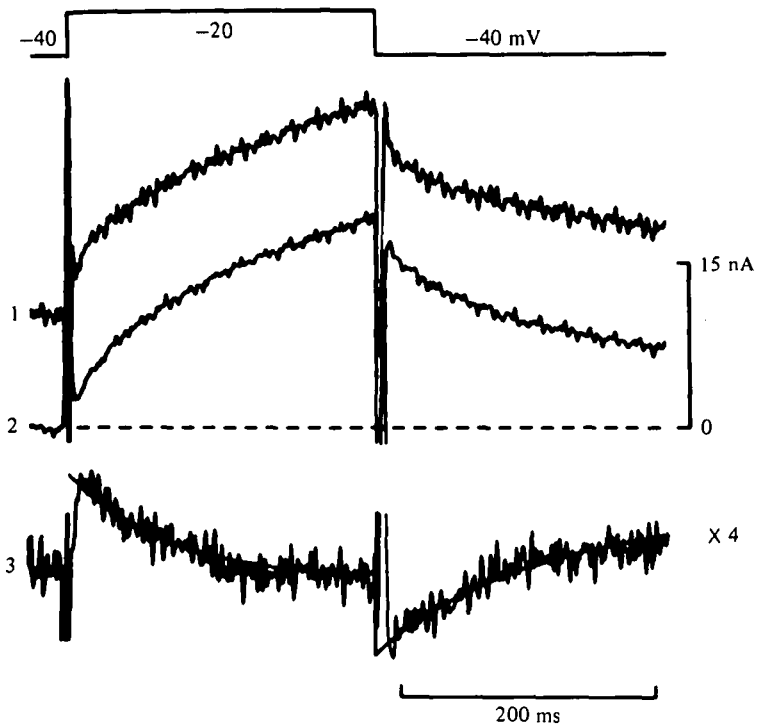

Fig. 14. Relaxation of acetylcholine-induced current in the SA node of rabbit. Current traces 2 and 1 (averages of several curves) were recorded during 20 mV voltage-clamp depolarizations before and during (respectively) the application of 2×10^{-6} M acetylcholine. Trace 3 shows the extra current elicited by ACh. Note the fourfold increase in amplification of trace 3. In 10^{-6} g/ml D600 and 10^{-7} g/m TTX. (Noma & Trautwein, 1978.)

In these experiments any simultaneous decrease in slow inward (Ca^{2+}/Na^{+}) current would be masked by the other current changes brought about by acetylcholine, and there are some observations which suggest that such a decrease in Ca^{2+}/Na^{+} current may occur in mammalian sinus. Paes de Carvalho *et al.* (1969, 1971) showed that the upstroke of action potentials from the atrium, bundle of His and the ventricle of the rabbit consists of a rapidly rising initial component followed by a slower component, while action potentials from the SA and AV nodes lack the initial fast phase. (This agrees with voltage-clamp findings of the distribution of rapid inward (Na^{+}) and slow inward (Ca^{2+}/Na^{+}) components.) They were able to show that focal application of acetylcholine depressed the slower component in atrial and 'transitional' nodal cells and abolished completely the action potentials of sinus cells in which the upstroke had consisted of a slowly rising phase only. This result is not the same as Kreitner's; it is possible that not all mammalian sinus cells have a fast sodium system which is revealed on acetylcholine application. The dose of acetylcholine is also uncertain when it is focally applied in this way.

Lipsius & Vassalle (1978) have recorded action potentials from 'subsidiary pacemakers' in the guinea-pig sinus node. The action potentials of these cells have both fast (Na^{+}) and slow (Ca^{2+}/Na^{+}) components in the upstroke and acetylcholine appears

to abolish the slow component while the fast component is, in association with hyperpolarization, increased.

Noma & Trautwein (1978) using the double microelectrode technique to voltage-clamp small preparations from the SA node of the rabbit have found that subtraction of currents recorded in the presence of acetylcholine ($2{\cdot}5 \times 10^{-6}$ M) from those recorded in normal Tyrode solution reveals a current that appears to decrease with time during depolarizing clamp pulses and to increase with time during hyperpolarizing pulses. The same result is obtained when D 600 and tetrodotoxin (both at concentration of 10^{-7} g/ml) are used in all solutions implying that this extra current is not due to a change induced by ACh on the slow or fast inward currents (Fig. 14). Noma & Trautwein interpret their results by proposing the activation by acetylcholine of a membrane channel which allows increased movement of potassium ions but which inactivates at positive membrane potentials and reactivates at negative membrane potentials, thus allowing such current changes to occur. This channel probably forms part but not all of the background i_{K_1} current (Noble, 1962).

Since the results of Noma and Trautwein were obtained using very small preparations dissected from the dominant pacemaker region of the rabbit sino-atrial node (see above) it seems probable that the primary action of acetylcholine in this tissue is to open channels which carry a potassium current. However, the single microelectrode work referred to above does suggest that in other regions of the sinus acetylcholine may also have a direct effect on the slow inward current. Since it is well known that the dominant pacemaker site is easily shifted by both cardio-active drugs and by temperature variations (Bouman *et al.* 1978; Steinbeck *et al.* 1978), it is difficult at the moment to assess precisely the relative importance of these two actions of acetylcholine in situ.

Actions of adrenaline on sino-atrial node

It has been known for some time that catecholamines increase the rate of the pacemaker depolarization and thus the frequency of spontaneous firing of sinus cells (Hutter & Trautwein, 1956; Hutter, 1957). However, because of the difficulties of applying the voltage clamp to sinus tissue (see above) the mechanism whereby such acceleration is brought about has not yet been fully explained.

A preliminary understanding of how adrenaline affects pacemaker cells can be obtained by observing carefully the changes it induces in their spontaneous activity. Such changes are shown in Fig. 15A, which was recorded from a small preparation of rabbit sino-atrial node of the type also used for voltage clamping. Perfusion with adrenaline (5×10^{-7} M) increases the rate of rise of the pacemaker depolarization and therefore the firing frequency. It also increases the overshoot of the action potentials, their rate of rise (dV/dt) and their rate of repolarization. There is also a small increase in the value of the maximum diastolic potential. Since the action potential upstroke in sinus cells can be mainly attributed to the activation of the slow inward (Ca^{2+}/Na^{+}) current (Yamagishi & Sano, 1966; Noma *et al.* 1977) the increased overshoot and rate of rise in adrenaline probably reflect an increase in the magnitude of this component. This is all the more probable since adrenaline is known to increase the magnitude of the slow inward current in other cardiac tissues (Vassort *et al.* 1967; Reuter & Scholz 1977*b*). On the other hand, the augmented maximum diastolic potential and rate

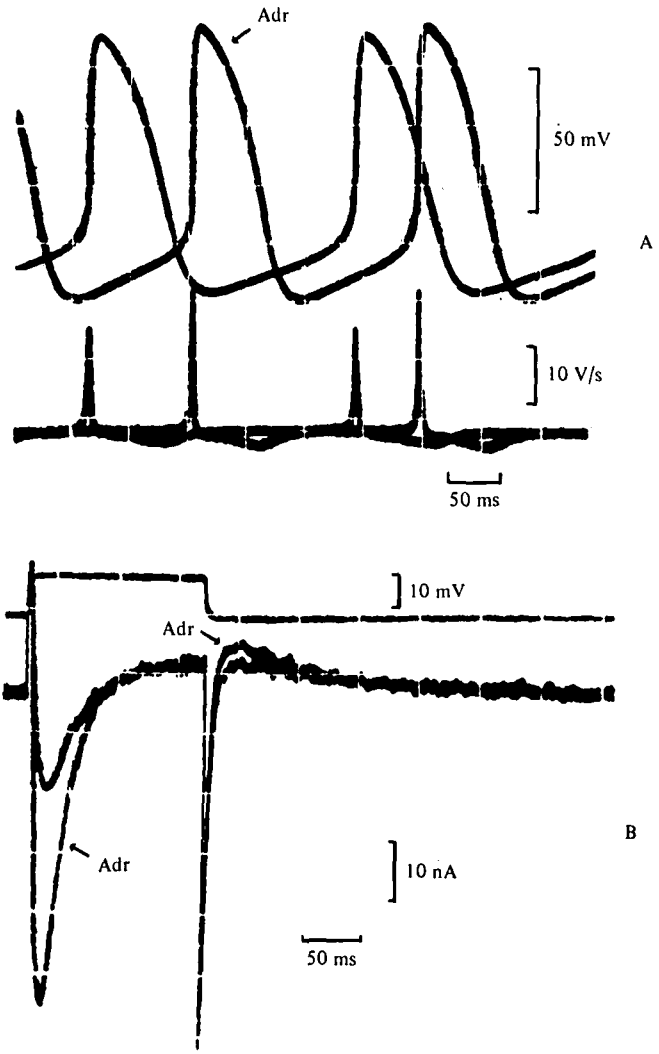

Fig. 15. Rabbit sino-atrial node; intracellular recording, in tetrodotoxin, 10^{-7} g/ml. (A) Voltage (above) and rate of change of voltage (below) before and during the action of adrenaline 5×10^{-7} g/ml. (B) Voltage clamp of same preparation: 10 mV clamp depolarizations from a holding potential of -45 mV before and during the action of adrenaline 5×10^{-8} g/ml. Note the increase in slow inward and in outward current.

repolarization imply that an outward, potassium current is also increased. However, from action potential configuration alone, it is impossible to distinguish between a direct effect of adrenaline on outward current and indirect extra activation due to the increase in overshoot potential.

Voltage-clamp analysis of the action of adrenaline on the SA node membrane currents

More reliable evidence about the nature of these changes comes from voltage-clamp experiments. In the experiment shown in Fig. 15 B the double microelectrode voltage-clamp technique was used. A 10 mV voltage-clamp depolarization given in the

presence of tetrodotoxin (10^{-7} g/ml) shows that the slow inward current is, as expected, greatly increased by adrenaline. It can also be seen in this record that the outward current tail after the depolarizing pulse is larger in adrenaline than it is in normal Tyrode. Such an increase in outward current is also found in frog atrium (Brown & Noble, 1974), in the sheep Purkinje fibre (Tsien, Giles & Greengard, 1972; DiFrancesco & McNaughton, unpublished) and in the tortoise sinus venosus (see Brown, Noble & Noble, 1978, fig. 15). Since this increase in outward current occurs with a small voltage-clamp depolarization, the result implies that the increased negativity of the maximum diastolic potential and the accelerated repolarization in adrenaline are caused by an increase in the amount of time-dependent outward current at all potentials rather than to a secondary effect produced by the augmented overshoot.

What mechanism produces the increase in outward current? The simplest possibility is that adrenaline has a direct effect on the outward current channel itself. Alternatively, adrenaline might increase outward current by stimulating the Na^+–K^+ exchange pump (Rogus, Cheng & Zierler, 1977), thus inducing depletion of potassium in close extracellular spaces and thereby increasing the driving force on potassium ions (Cohen, Eisner & Noble, 1978). Yet another possibility is suggested by some preliminary experimental results obtained in our laboratory. We have observed that the presence of the slow inward channel blocker, D600, at a concentration of 10^{-7} g/ml causes a decrease in both slow inward and outward currents, while raised calcium concentration does the opposite.

It would therefore seem possible that there is a calcium activated potassium conductance mechanism in the mammalian SA node (cf. Meech, 1972). In this case the action of adrenaline on i_p would be an indirect effect of the increase induced in the slow inward current.

How does adrenaline change the frequency of spontaneous pacemaking?

While an adrenaline induced increase in outward current would explain the more hyperpolarized maximum diastolic potential and the faster repolarization of the action potential, it could not explain the acceleration of the pacemaker depolarization observed in adrenaline (Fig. 15A). In fact a maintained increase in outward current would by itself cause a slower rate of pacemaker depolarization, unless the kinetics of the outward current were also modified. The large increase in the slow inward current which is observed in adrenaline could however contribute to at least part of the pacemaker potential thus increasing its rate of rise as has been shown to occur for induced pacemaking in the frog atrium (Brown & Noble, 1974). Further acceleration could be produced by an increase in the inward time-independent background current but it seems unlikely that this occurs as no evident inwardly directed change in the steady current at the holding potential is observed when adrenaline is applied (Fig. 15B).

It is interesting that the additional current change, i_f, which is normally activated in sinus at potentials more negative than -50 to -60 mV (see above) is greatly increased in the presence of adrenaline. This is shown in Fig. 16, where the current during hyperpolarizing pulses of 500 ms from a holding potential of -36 mV are

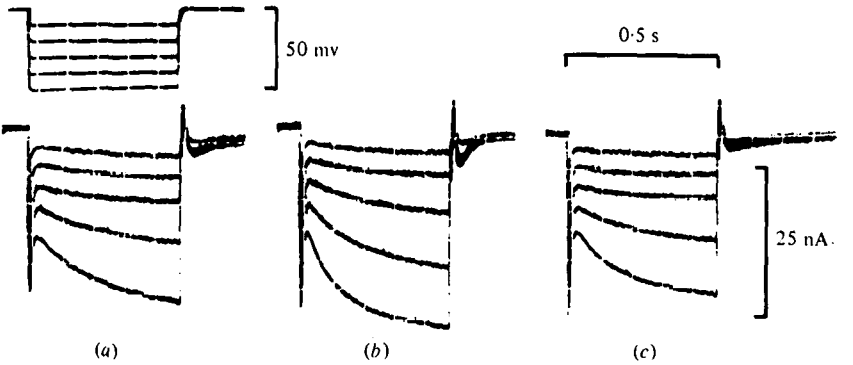

Fig. 16. Rabbit sinoatrial node. Effect of adrenaline on the additional current, i_f. Hyperpolarizing voltage-clamp pulses were applied from the holding potential of −36 mV before (*a*), during (*b*) and after (*c*) perfusion with adrenaline 10^{-7} M. Note that the time-dependent current change was markedly increased by adrenaline during the −30, −40 and −50 mV hyperpolarizations and to a smaller extent during the −20 mV pulse. In TTX (10^{-7} M) and D600 (10^{-7} M).

compared before and during adrenaline action. The properties and function of this current are not yet understood but the result shown in Fig. 16 suggests that i_f could play an important role in modulating the effect of adrenaline on the slow diastolic depolarization. Certainly, if the potential range in which the increase in this current occurs overlaps with the pacemaker depolarization then i_f would definitely contribute towards acceleration.

APPENDIX

Exponential analysis of outward current in the presence of potassium accumulation

A problem which concerns many cardiac electrophysiologists is whether it is possible to analyse satisfactorily the various components of outward membrane current in the presence of accumulation or depletion of potassium ions in restricted, extracellular spaces. Such accumulation of potassium ions will induce changes in the potassium equilibrium potential across the membrane which is known to distort both time-dependent currents and the time-independent background current first described by Noble in 1962 (see also Maughan, 1973; McGuigan, 1974; Brown, Clark & Noble, 1976*a*, *b*; Noble, 1976; Baumgarten & Isenberg, 1977; DiFrancesco & Ohba, 1978; DiFrancesco, Ohba & Ojeda (1979); Eisner, Cohen & Attwell (1980)). (The papers cited deal with other cardiac tissues (ventricle, atrium and Purkinje fibres) but the problems are essentially the same in the analysis of the sinus currents.) It might even be suggested that all the time-dependent outward current is produced by potassium accumulation resulting from potassium movement through time-independent channels. It is possible to sustain or refute such a suggestion by experimental means and this we have done using the frog atrial preparation described in Brown, Clark & Noble, 1976(*a*).

Is time-dependent outward current present in cardiac muscle?

(1) *The theoretical approach.* In any cardiac membrane several currents can be presumed to flow at a given potential. Suppose we let i_{K_1} be the potassium current that is not controlled by gating mechanisms and is therefore generally called time-independent. The magnitude of this current will be a function of the membrane potential, E, and of potassium concentration in close extracellular spaces, K_c. Note, however, that it is also an implicit function of time through the time dependence of K_c. Now let i_x be any time-dependent current controlled by Hodgkin–Huxley kinetics. This current is an explicit function of E, of time, t, and of K_c. The net current carried by the Na–K exchange pump, i_{pump}, must also be taken into consideration. We assume that its magnitude depends only on K_c.

If the perfusing solution contains tetrodotoxin to block the fast inward (Na^+) current, and manganese to block the slow inward (Ca^{2+}/Na^+) current, the remaining inward current, i_{in}, will not interfere appreciably with the measurement of outward current and will anyway be independent of K_c.

The general equation for the total ionic current will then be:

$$i_i(E, t, K_c) = \bar{i}_x(E, K_c).x(E, t)+i_{K_1}(E, K_c)+i_{\text{pump}}(K_c)+i_{\text{in}}(E), \tag{1}$$

where $\bar{i}_x$ is the fully activated current carried by the x channels when all are open and x describes the fraction of channels in the open state. Our question is: can we show that i_x must exist?

Let us consider 'on' instantaneous current–voltage relations obtained by voltage clamping from the resting potential, E_H, at which K_c has its resting value K_{c_1} and x has its resting value x_1 to any potential E (see inset A, Fig. 7). Then the current jump is given by

$$i_{\text{on}_1} = \bar{i}_x(E, K_{c_1}).x_1+i_{K_1}(E, K_{c_1})+i_{\text{pump}}(K_{c_1})+i_{\text{in}}(E) \tag{2}$$

(i_{on} is measured by extrapolation as shown in inset A, Fig. 7).

Now let a potential E_T be held for a period of time, t, during which K_c increases from K_{c_1} to K_{c_2} and x increases from x_1 to x_2. An 'off' instantaneous current–voltage relation may now be obtained by clamping from E_T at t to a series of potentials E (see Fig. 7, inset B). The current at the beginning of the clamp to voltage E referred to the zero current level will be given by

$$i_{\text{off}} = \bar{i}_x(E, K_{c_2}).x_2+i_{K_1}(E, K_{c_2})+i_{\text{pump}}(K_{c_2})+i_{\text{in}}(E). \tag{3}$$

Supposing the potassium concentration of the perfusing solution is now increased so as to change K_c to K_{c_2}, then, holding at the new resting potential, we can obtain a second 'on' instantaneous relation such that

$$i_{\text{on}_2} = \bar{i}_x(E, K_{c_2}).x_1'+i_{K_1}(E, K_{c_2})+i_{\text{pump}}(K_{c_2})+i_{\text{in}}(E), \tag{4}$$

where x_1' is the new resting value of x. Clearly (3) and (4) differ only by the current

$$i_{\text{off}}-i_{\text{on}_2} = \bar{i}_x(E, K_{c_2}).(x_2-x_1') = \Delta i_x. \tag{5}$$

Thus, if no x current exists (3) and (4) will be identical and the current–voltage diagrams obtained by changing K_c to the same value, either by depolarization ('off

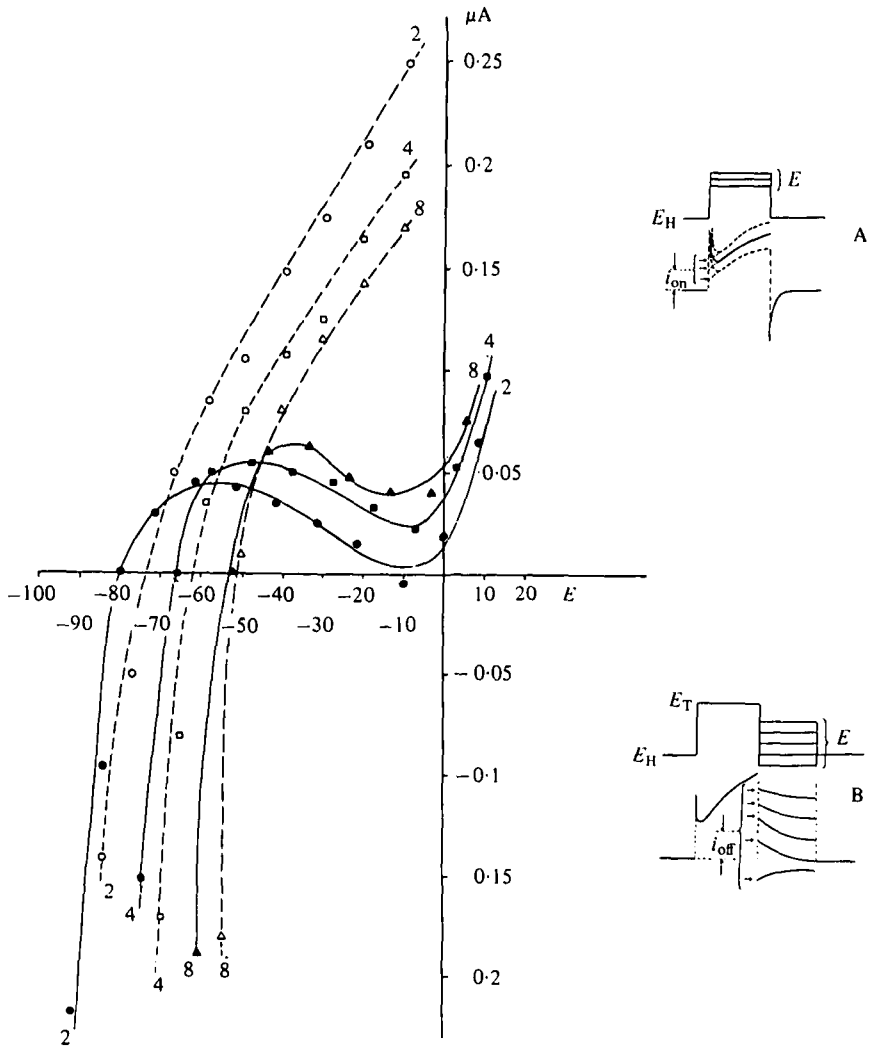

Fig. 17. Current–voltage relationships of frog atrial preparation obtained in 2 mM (circles), 4 mM (squares) and 8 mM-K^+ (triangles). Open symbols: 'on' relationships obtained as shown in inset A. Filled symbols: 'off' relationships obtained as shown in inset B. See text.

relation) or by increasing the external potassium concentration, K_b ('on' relation) will superimpose. If the experimental 'on' and 'off' relations do not superimpose then some i_x current must exist.

(2) *The experimental result.* A set of experimental 'on' (solid lines) and 'off' (broken lines) current–voltage relations are shown in Fig. 17. In practice it is very difficult to achieve a situation in which K_c is identical for a given pair of curves. In the case of the 'on' relations, K_c is increased by increasing the value of the bulk extracellular potassium concentration, K_b, and this bears a non-linear relationship to K_c, which is difficult to determine exactly. Again, in the case of the 'off' relations there is no way of determining how much accumulation will take place during the pulse to E_T for time t and therefore no way of accurately assessing the resulting value of K_c. Nevertheless, it

is quite clear from Fig. 17 that the 'on' and 'off' current–voltage relations are not only very far from superimposing but also behave differently as K_c is increased. In the case of the 'on' relations (filled circles, K_b = 2 mM; filled squares, K_b = 4 mM and filled triangles, K_b = 8 mM) the curves show the 'cross-over' phenomenon characteristic of current–voltage relationships for time-independent outward current (see Noble, 1965): the outward current increases between −60 and +10 mV with increasing K_c. By contrast, the 'off' relations (open circles, K_b = 2 mM; open squares, K_b = 4 mM and open triangles, K_b = 8 mM) although all showing a greatly increased component of outward current, when compared with any of their 'on' counterparts nevertheless show no cross-over, the outward current decreasing in magnitude with increasing K_c. From this we can conclude that not only do membrane channels carrying time-dependent i_x current exist but that these channels have entirely different properties from those which carry the time-independent outward current, i_{K_1}.

The effects of K^+ accumulation on outward currents

The experiment whose results are shown in Fig. 17 not only demonstrates that time-dependent outward current channels exist in cardiac muscle, it also shows that potassium accumulation in extracellular spaces must accompany the flow of such currents (cf. Kronhaus *et al.* (1978)). It is this accumulation that causes the reversal potentials for the 'on' and 'off' curves obtained in the same external potassium concentration to differ. A very important problem is how can the various time-dependent outward currents which are generated by depolarization be separated from one another and from the effects of accumulation? The potassium ion movement occurring during and after voltage-clamp depolarization will affect the outward currents in several different ways. In an attempt to separate these various effects, a numerical model has been set up based on our experimental data (Brown, DiFrancesco, Noble & Noble, 1980; DiFrancesco & Noble, 1980). Fig. 18 shows a decay tail (filled circles) obtained using the numerical model at a holding potential of −40 mV after a pulse of +30 mV for 5 s. At the beginning of the decay tail the time-dependent current, i_x, still flows, for it takes a certain time to de-activate. If K_c has not reached a steady-state during the preceding pulse it is possible therefore that K_c will at first continue to increase. As is shown in Fig. 18, the time course of the time-independent outward current will in this case increase (crosses) before decaying exponentially (later part of the total decay tail).

The exponentially decaying portion of the curve can be extrapolated to zero time and then subtracted from the total current to give i_x (open circles in Fig. 18). The error in this method of estimating i_x, indicated by the shaded portion in Fig. 18, and separately plotted as open triangles, will reduce the amplitude of i_x. If it is compared with the estimated i_x (open circles) it can be seen that though it would somewhat reduce the magnitude of i_x, it would hardly affect its time constant of decay.

The computed decay tail in Fig. 18 is in one way oversimplified, for it contains only one time-dependent component of outward current. However, in both the frog sinus and frog atrium we have consistently found that two time-dependent outward current components are present. Fig. 19 shows semi-logarithmic plots of two decay tails recorded from a frog sinus preparation at a holding potential of −50 mV after depolarizing pulses of +60 mV for 300 msec and +30 mV for 7 s. After removal of an accumulation component due to the effect of K_c on $i_{K_1}+i_{\text{pump}}$ (τ = 7·5 s), two

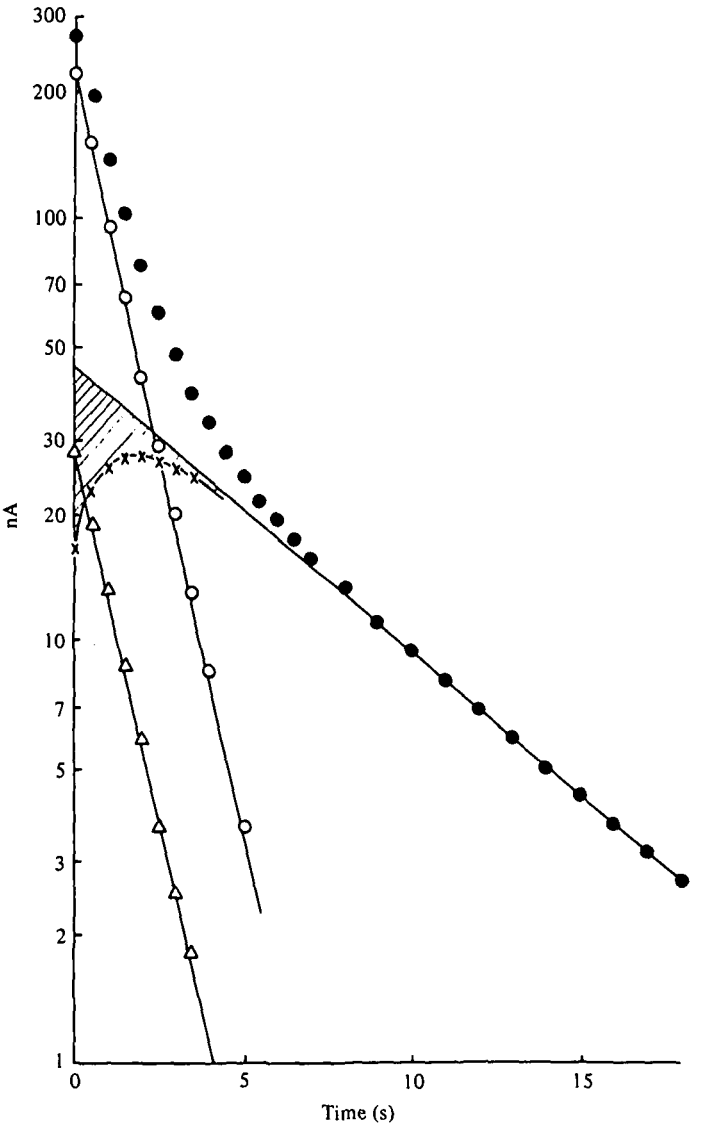

Fig. 18. Analysis of a current decay tail obtained using the numerical model mentioned in the text after a simulated voltage-clamp pulse of 30 mV for 5 s from a holding potential of −40 mV. The tail has been plotted semi-logarithmically (●). The later, exponentially decaying part of the tail (attributable primarily to potassium depletion) has been extrapolated to zero time and subtracted from the total current to give the time course of the decay of time-dependent outward current, i_x (○). The time-course of time-independent outward current at the start of the tail when i_x is still flowing and K_c consequently still increasing is shown by the crosses. The error resulting from this is indicated by the shaded area and is separately plotted (△, obtained by subtracting the crosses from the (extrapolated) potassium depletion component line). It is evident that this error would affect the magnitude of i_x separated in this way by approx. 15% but leave the time constant of i_x decay virtually unaltered.

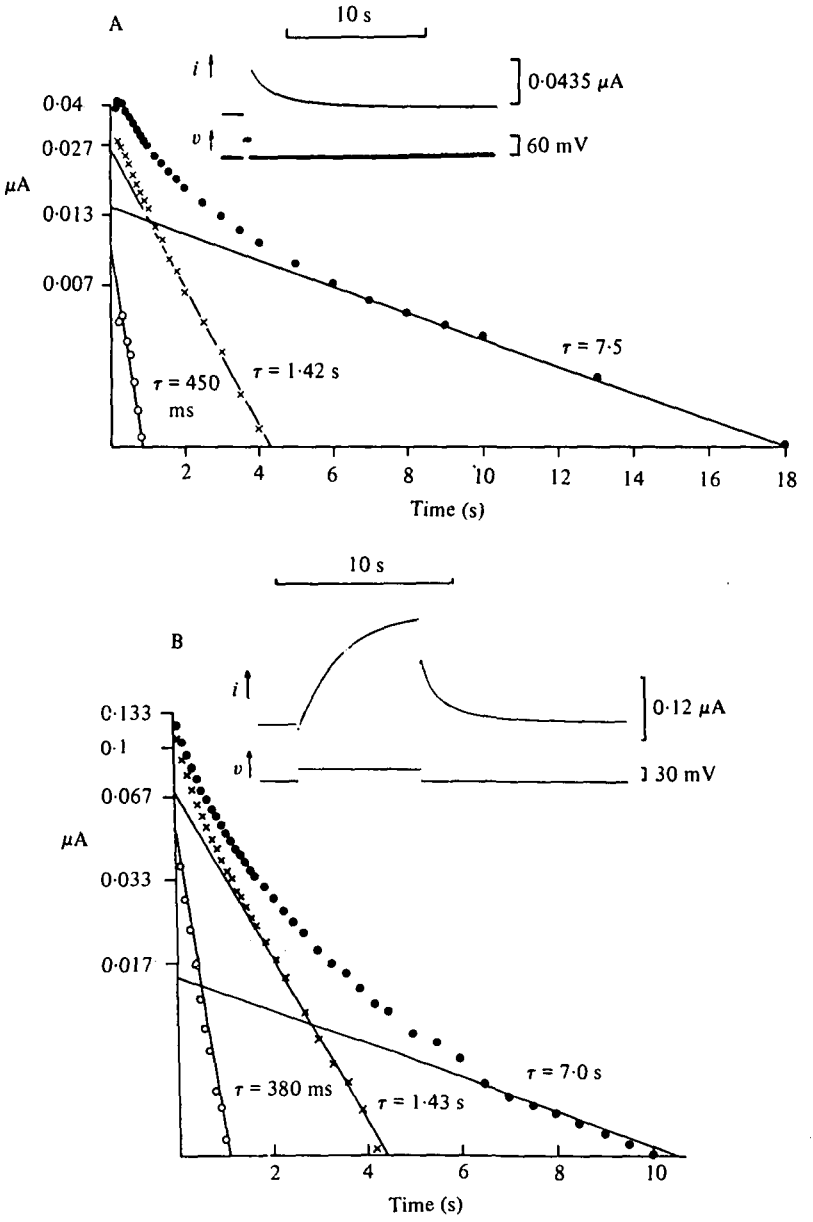

Fig. 19. Frog sinus preparation; double sucrose-gap technique. Current decay tails recorded at −50 mV after voltage-clamp depolarizing pulses of +60 mV for 300 ms (above) and +30 mV for 7 s (below) were plotted on a semilogarithmic scale and separated into exponentially decaying components. After removal of the slowest (accumulation) component, two other components with time constants of 1·4 s and about 400 ms can be separated.

other components can be separated. The time constant of the more slowly decaying component of time-dependent outward current (1·4 s) is such that this component can underlie pacemaker depolarizations. The function of the faster-decaying component (τ = *ca.* 400 msec) is more difficult to understand but it does not seem to be an artifact either in frog sinus or in frog atrium (Brown, Noble & Noble, 1976; DiFrancesco & Noble, 1980).

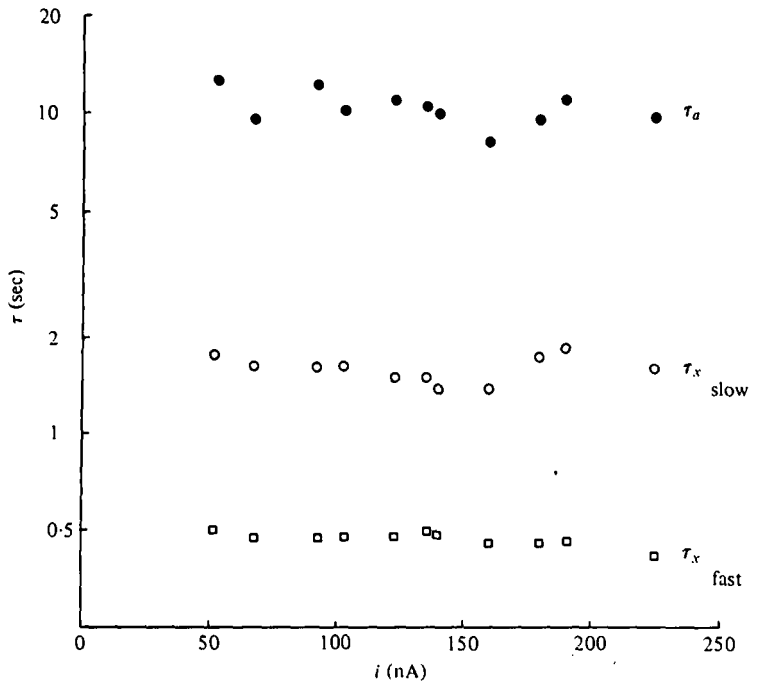

Fig. 20. Values of τ_a, τ_{slow} and τ_{fast} all obtained at the same holding potential of 40 mV over a wide range of tail amplitudes in an experiment on frog sinus (see Brown, Giles & Noble, 1977*b*). The time constant values were obtained from the experimental results using the exponential fitting programme described by Provencher (1976). Note that there is no large systematic variation of the time constants with tail amplitude and, hence, with the degree of activation or magnitude of potassium accumulation.

Fig. 20 shows that in frog sinus the time constants of the three components, i_{fast}, i_{slow} and $i_{accumulation}$ are remarkably constant over a wide range of tail amplitudes when separated by semi-logarithmic (exponential) analysis. The data shown were obtained by hand but were checked using a Fourier method for the analysis of exponential decay curves (Provencher, 1976).

Using such exponential splitting, activation curves can be plotted for the fast and for the more slowly decaying outward current components. How accurate are such activation curves? We have shown above (Fig. 18) that the time constants of decay of components separated in this way are likely to be accurate whereas their magnitude will be somewhat underestimated. This means that the overall magnitude of a given activation curve plotted from such separated components will be reduced in size but this is not as important as its overall position on the voltage axis and its general shape. That these are relatively unchanged by the splitting technique is also shown by DiFrancesco & Noble (1980) using an analytical solution for a three-compartment model. Their result is illustrated in Fig. 21, which shows that if the increase of K_c with the increase of the degree of activation x during a depolarization is linear (ΔK_c = 10 Δx mM), there is only a 6 mV shift in the position of the normalized activation curve on the voltage axis. Under normal experimental conditions ΔK_c is likely to be less than 10 Δx mM and the normalized activation curves obtained would therefore be expected to give an even better indication of the true picture.

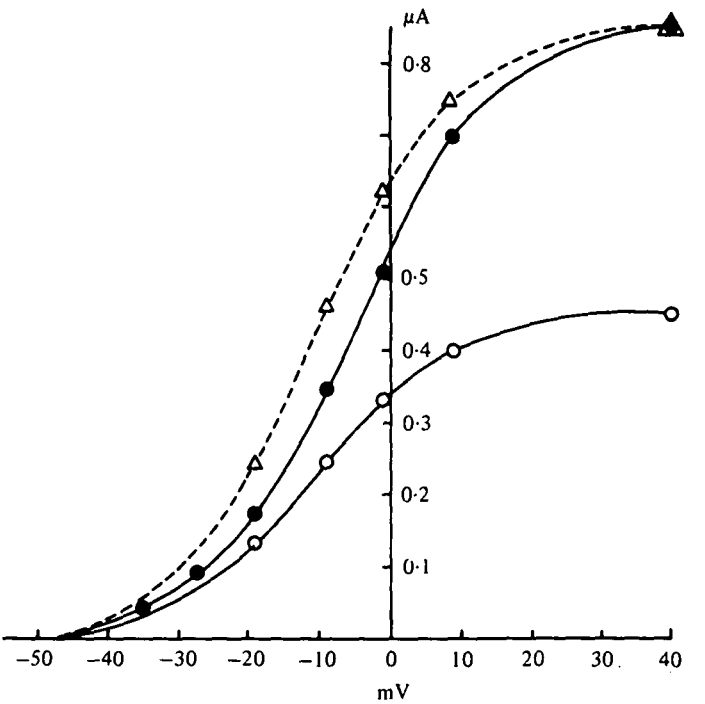

Fig. 21. Effect of cleft potassium accumulation/depletion processes on the activation curve for the outward current in frog atrium as calculated from a numerical, 3-compartment model (Brown, DiFrancesco, Noble & Noble, in preparation). ●, 'True' activation curve, unaffected by accumulation. When accumulation is allowed for during the voltage-clamp depolarizations, the current tails succeeding them are smaller and therefore the activation curve constructed from them is smaller also (○). If this latter activation curve is scaled up (△) to compare with the true curve, the shift in the half activation point is only −6 mV.

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