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NORADRENALINE HYPERPOLARIZES CELLS OF THE CANINE CORONARY SINUS BY INCREASING THEIR PERMEABILITY TO POTASSIUM IONS

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SUMMARY

1. The mechanism of the noradrenaline-induced hyperpolarization was investigated in small strips of coronary sinus tissue mounted in a fast-flow system.

2. The recorded hyperpolarization was negligibly small in response to 10 nM-noradrenaline but was maximal at 10 μM (average amplitude 23 mV, in 4 mM-K solution). The hyperpolarization was unaffected by 1 μM-phentolamine but was abolished by 10 μM-propranolol and so is presumably mediated via β-adrenoceptors.

3. The noradrenaline-induced hyperpolarization became smaller when the extracellular K concentration ($[K]_o$) was raised or when the extracellular Na concentration was lowered.

4. These results are consistent with two general mechanisms: noradrenaline might cause hyperpolarization by stimulating the Na/K pump to generate more outward current, as previously suggested for other cell types. Alternatively, noradrenaline might lower the permeability ratio, P_{Na}/P_K, by reducing the permeability coefficient for Na (P_{Na}) and/or increasing that for K (P_K).

5. The noradrenaline-induced hyperpolarization is not diminished during exposure to 5 μM-acetylstrophanthidin, or to K-free solution, or to K-free solution containing acetylstrophanthidin. We conclude that the hyperpolarization does not reflect enhanced electrogenic pump activity.

6. Conductance measurements using two micro-electrodes in very small preparations revealed that, like the muscarinic agonist carbachol, noradrenaline caused an increase in membrane slope conductance. Steady-state current–voltage curves obtained in the presence of noradrenaline, in the presence of carbachol, and in the absence of both drugs all crossed each other at about the same level of membrane potential. During the maintained injection of sufficiently large hyperpolarizing current, application of either noradrenaline or carbachol causes depolarization instead of hyperpolarization.

7. The cross-over or 'reversal' potentials of current–voltage curves, determined with and without the drugs, vary with $[K]_o$ approximately as does the K equilibrium potential calculated assuming the intracellular K concentration to be 155 mM. We conclude that, like carbachol and acetylcholine, noradrenaline causes a specific increase in the K permeability of coronary sinus cells.

INTRODUCTION

It has long been known that exposure to adrenaline can cause marked hyperpolarization of atrial cells or of cardiac Purkinje cells that display a low resting potential (Dudel & Trautwein, 1956; Trautwein & Schmidt, 1960; Hoffman & Singer, 1967). During studies of triggered activity in the canine coronary sinus, Wit & Cranefield (1977) found that depolarized and inexcitable cells would, if exposed to noradrenaline, hyperpolarize and regain excitability. The hyperpolarization induced by adrenaline and noradrenaline is usually attributed to enhancement of the rate of active Na/K exchange (Trautwein & Schmidt, 1960; Akasu, Ohta & Koketsu, 1977; Wit & Cranefield, 1977) whereas the hyperpolarization of atrial fibres induced by acetylcholine has been clearly demonstrated to result from an increase in the permeability of the membrane to K ions (for review, see Hutter, 1964).

As shown in the preceding paper, quiescent cells of the canine coronary sinus that show a low resting potential when exposed to a normal extracellular concentration of K ions ($[K]_o$), i.e. about 4 mM, can be markedly hyperpolarized by exposure to acetylcholine. The low resting potential of those cells therefore results not from a low level of intracellular K concentration ($[K]_i$) but from the fact that the ratio of the permeability of the membrane to K ions (P_K), to that for Na ions (P_{Na}), is smaller than it is, for example, in Purkinje cells. Therefore, a possible alternative to enhanced Na/K pump activity as the mechanism of the noradrenaline-induced hyperpolarization of coronary sinus cells is that noradrenaline might cause an increase in the ratio P_K/P_{Na}, either by causing a selective increase in P_K, as acetylcholine does, or by causing a reduction in P_{Na}. We report here experiments that indicate that noradrenaline causes a specific increase in the K permeability of the membrane of coronary sinus cells. Because this conclusion is at such variance with prevailing opinion and with our own expectations, we have examined the effect of noradrenaline on coronary sinus cells over a wide range of membrane potentials, of levels of $[K]_o$, and of extracellular Na concentration ($[Na]_o$), and after inhibiting the Na/K pump by exposing preparations to the cardiac steroid acetylstrophanthidin and/or K-free solution; we have, further, compared the effects of noradrenaline and the cholinergic agonist, carbachol, on the membrane conductance of small preparations of coronary sinus cells.

METHODS

The methods used in these experiments were virtually identical to those described in the preceding paper (Boyden, Cranefield, Gadsby & Wit, 1983). Additional experimental details are given below in the text and Figure legends. L-Noradrenaline bitartrate (Levophed, Breon Laboratories, Sterling Drug Inc., NY) was added to superfusion solutions from a stock solution of concentration 1 mg/ml to produce the required uniform concentrations. Some preliminary tests were also made with L-adrenaline HCl (Parke, Davis & Co., Detroit, MI) and with L-isoproterenol bitartrate (Sigma Chemical Co., St. Louis, MO). To help prevent oxidation of the catecholamines, 10 μM-Na_2EDTA was added to all superfusing solutions (Furchgott, 1955). The β-adrenoceptor antagonist, DL-propranolol HCl (Sigma), and the α-adrenoceptor antagonist, phentolamine HCl (Regitine; Ciba-Geigy Corp., Summit, NJ), were added from concentrated stock solutions as required. In some experiments in which coronary sinus cells were depolarized with high K solutions, 1–2 μM racemic verapamil (kindly provided by Knoll AG, Ludwigshafen, F.R.G.) was added to prevent possible occurrence of bursts of triggered activity (Wit & Cranefield, 1977; Wit, Cranefield & Gadsby, 1981).

In the experiments to study the effect of noradrenaline on membrane conductance, a digital timing circuit was used to trigger the delivery of constant-current pulses, of variable amplitude and duration, to the current-injecting micro-electrode.

RESULTS

The superimposed records in Fig. 1 *A* were obtained during a maintained impalement of a quiescent coronary sinus strip exposed to 4 mM-K, low Cl solution, and they illustrate the graded hyperpolarizations caused by sudden application of five concentrations of noradrenaline, ranging from 10 nM to 100 μM. In the absence of

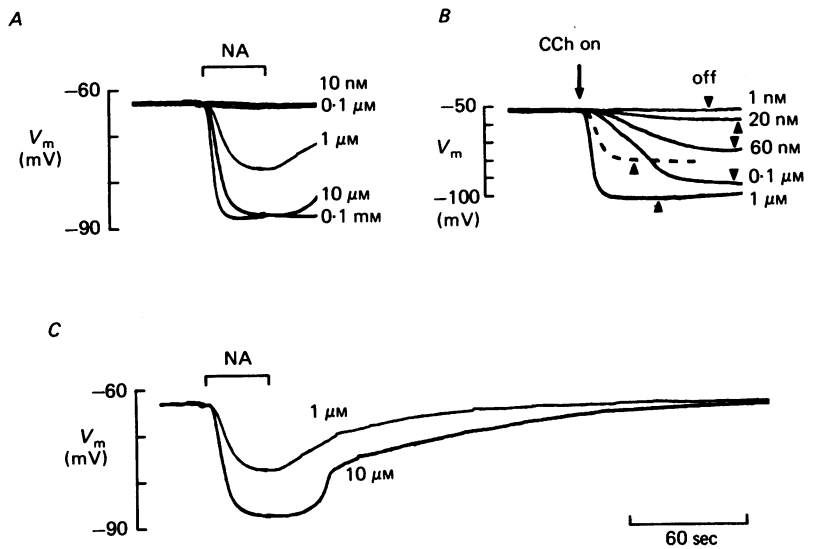

Fig. 1. Photographically superimposed chart recordings of changes in the membrane potential of small coronary sinus preparations, exposed to 4 mM-K, low Cl solutions, caused by brief applications of noradrenaline (NA) (*A*, *C*), or of carbachol (*B*), at the concentrations indicated beside each record. Noradrenaline was applied for the period indicated by the horizontal bars in *A* and *C*; the records in *C* illustrate the full time courses of the responses to 1 μM and 10 μM-noradrenaline shown in *A*. The records in *B* were obtained from another preparation; note the different voltage scale. Carbachol was applied at the time indicated by the downward arrow (labelled: CCh on) and was removed at the times indicated by the arrowheads. The dashed trace in *C* shows, for comparison, the response of that preparation to a brief application of 10 μM-noradrenaline. The time scale at the lower right applies to all traces.

noradrenaline, the resting potential of this preparation remained steady at −63 mV. The hyperpolarizations caused by 10 and 100 nM noradrenaline were negligibly small, whereas the 26 mV hyperpolarization caused by 10 μM-noradrenaline was of apparently maximal amplitude (i.e. closely similar to the hyperpolarization caused by 100 μM). Fig. 1 *B* shows superimposed records of the changes in membrane potential of another preparation exposed to 4 mM-K, low Cl solution, caused by sudden application of graded concentrations (1 nM to 1 μM) of the muscarinic cholinergic agonist, carbachol. In that preparation, carbachol caused graded hyper-

polarizations that were very small at concentrations of 1 and 20 nM, but approximately 50 mV in amplitude at 1 μM. For comparison, the dashed trace in Fig. 1*B* shows the hyperpolarization caused by 10 μM-noradrenaline in the same preparation and under the same conditions: as confirmed in many other similar experiments, the hyperpolarization caused by a maximal concentration of carbachol was substantially larger and was attained more rapidly than that caused by a maximal concentration of noradrenaline.

The time course of the noradrenaline-induced hyperpolarization was usually complex; at moderately low concentrations, it was clearly sigmoid in shape and, as can be seen in Fig. 1*A*, the rate of increase of membrane potential was greater at higher noradrenaline concentrations. Fig. 1*B* shows that the time course of the carbachol-induced hyperpolarization was similarly, roughly sigmoid at moderately low concentrations, but more rapid and more complex in shape at higher concentrations (e.g. 0·1 μM). The full time courses of noradrenaline-induced hyperpolarization, and of the subsequent depolarization on washing out the drug, are illustrated by the records in Fig. 1*C*. During the 30 sec exposure to either 1 μM or 10 μM-noradrenaline, the membrane potential increased along a sigmoidal time course to an approximately steady level. However, the subsequent decline in potential on washing out the noradrenaline, although occurring with a relatively simple time course (after a short delay) in the case of the lower concentration, had a much more complex shape in the case of the 10 μM concentration: the potential declined at first slowly, then abruptly, and then again more slowly. The abrupt change in membrane potential occurred over about the same voltage range as similar abrupt potential changes seen, for example, during the depolarization caused by the wash-out of high concentrations of acetylcholine or carbachol, or during the hyperpolarization caused by lowering the extracellular Na concentration, or during the hyperpolarization caused by a ramp of slowly increasing hyperpolarizing current and, presumably, these abrupt changes all reflect the marked non-linearity of the steady-state membrane current–voltage relationship of quiescent coronary sinus cells (see, e.g. Figs. 3, 5 and 8 of Boyden *et al.* 1983).

The dose–response plot of Fig. 2 summarizes average results obtained from seven preparations: in each experiment, the amplitude of the steady hyperpolarization elicited by application of a given concentration of noradrenaline (1 nM to 100 μM) has been normalized with respect to the mean size of the hyperpolarization induced in that same preparation by applications of 10 μM-noradrenaline. Usually, each concentration of noradrenaline was tested at least three times in each preparation (except 100 μM, which was tested only once in each preparation because the subsequent recovery was slow), so that a mean normalized hyperpolarization could be determined for each dose. These mean values have been averaged over all seven experiments to obtain each point plotted in Fig. 2. The over-all average size of the hyperpolarization caused by 10 μM-noradrenaline in these experiments, in 4 mM-K low Cl solution, was 23 mV (S.D. $\pm$ 7 mV). The smooth curve drawn through the points has the form of a saturable, one-to-one binding curve. The reasonable fit of that curve to the experimental data might be interpreted as indicating that the size of the noradrenaline-induced hyperpolarization is directly proportional to the fraction of the population of binding sites occupied by noradrenaline, with half-maximal

occupancy occurring at a noradrenaline concentration of 0·4 μM. However, there are a number of reasons for believing that such an interpretation is likely to be greatly oversimplified (see Discussion). Nevertheless, it probably *is* reasonable to conclude (*a*) that the minimum noradrenaline concentration required to cause a measurable hyperpolarization lies between 10 and 100 nM, and (*b*) that, for practical purposes, 10 μM-noradrenaline is a maximally effective concentration.

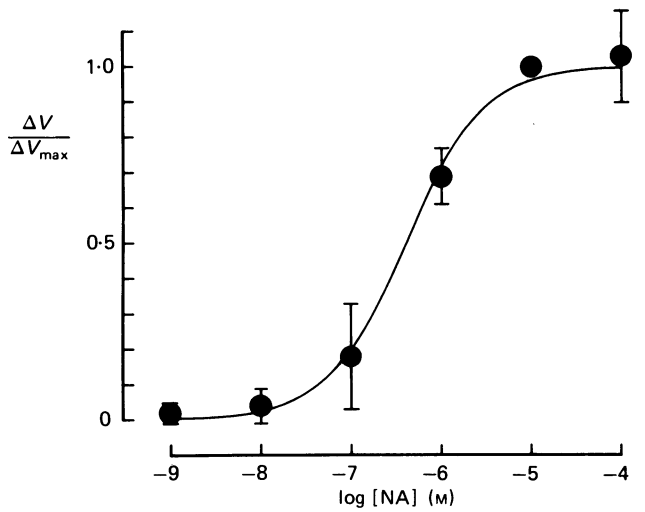

Fig. 2. Normalized dose–response plot of the noradrenaline-induced hyperpolarization. The steady hyperpolarization, $\triangle V$, induced by each concentration of noradrenaline was normalized to the mean hyperpolarization elicited in the same preparation by 10 μM-noradrenaline, $\triangle V_{max}$. The graph summarizes normalized results averaged over seven preparations: the vertical bars indicate $\pm$S.D. The curve is drawn according to $\triangle V/\triangle V$ max = [NA]/([NA]+$K_{0·5}$), where $K_{0·5}$ represents the noradrenaline concentration at which $\triangle V = 0·5\ \triangle V_{max}$, and is in this case 0·4 μM.

Preliminary results from three experiments in which the noradrenaline-induced hyperpolarization of coronary sinus cells was compared with the similar hyperpolarizations caused by application of adrenaline (one preparation) or isoproterenol (two preparations), over a wide range of concentrations, suggested that all these catecholamines are roughly equipotent in this regard, with half-maximal effects being caused by exposure to 0·2–1·0 μM-catecholamine.

The records in Fig. 3 demonstrate the effects on the noradrenaline-induced hyperpolarization of exposing a coronary sinus preparation to a high concentration of either phentolamine, an antagonist of α-adrenoceptors or propranolol, an antagonist of β-adrenoceptors. The upper record in Fig. 3*A* was obtained from a coronary sinus strip superfused with Tyrode solution containing 1 mM-K and 147 mM-Cl, and shows the progressive increase in membrane potential caused by exposure to noradrenaline concentrations that were increased in a stepwise manner, from 0·1 to 1 μM and then to 10 μM. After several minutes of washing out the noradrenaline, the membrane potential had returned to its steady control level, of −65 mV, and 1 μM-phentolamine

was then added to the superfusate. After 5 min, the phentolamine had caused no change in membrane potential and application of the three noradrenaline concentrations gave rise to a progressive hyperpolarization (not shown here) identical to that obtained under control conditions. We then washed out the noradrenaline while maintaining the phentolamine concentration for a further 20 min to ensure that any action would indeed be complete, and then again tested the three noradrenaline

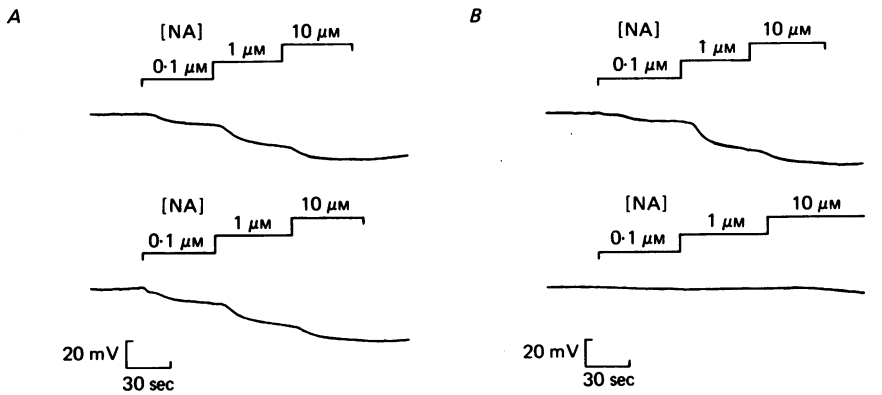

Fig. 3. Effects of adrenoceptor antagonists on the noradrenaline-induced hyperpolarization. The timing of the exposures to noradrenaline and of the stepwise increases in noradrenaline concentration are indicated above each voltage record. The upper records in *A* and *B* show the control responses, and the lower records show the responses obtained in the presence of 1 μM-phentolamine HCl (*A*), and in the presence of 10 μM-propranolol HCl *B*, respectively. 1 mM-K, normal Cl-containing solutions throughout; the steady resting potential in the absence of noradrenaline was −65 mV in both records in *A* and was −64 mV in both records in *B*.

concentrations still in the presence of phentolamine as shown in Fig. 3*A*, lower trace: it is evident that 1 μM-phentolamine has no effect on the noradrenaline-induced hyperpolarization. The upper record in Fig. 3*B* was obtained from the same preparation some 2 hr later (in the absence of phentolamine) and again shows the steplike increases in membrane potential caused by stepwise increases in the concentration of noradrenaline. Application of 10 μM-propranolol almost 20 min after washing out the noradrenaline, when the membrane potential had returned to its control level, caused no measurable change in potential. However, the lower record in Fig. 3*B* was obtained 3 min later and shows that, in the presence of propranolol, application of noradrenaline caused practically no change in the membrane potential. Similar experiments revealed that the noradrenaline-induced hyperpolarization was unaffected by the presence of 10 μM-atropine, a concentration of the muscarinic antagonist sufficient to abolish the hyperpolarization caused by acetylcholine or carbachol.

We conclude from these results that the noradrenaline-induced hyperpolarization is mediated by activation of β-adrenoceptors, and is thus pharmacologically quite distinct from the acetylcholine-induced hyperpolarization which is mediated by activation of muscarinic cholinergic receptors. However, these two kinds of drug-induced hyperpolarization do have several important features in common. For

example, as we reported in the preceding paper (Boyden *et al.* 1983), the amplitude of the ACh-induced hyperpolarization is reduced as the extracellular K concentration, $[K]_o$, is increased; the results in Fig. 4 show that the same is true of the noradrenaline-induced hyperpolarization. The records in Fig. 4*A* illustrate changes in resting potential caused by brief applications of a maximally effective concentration of

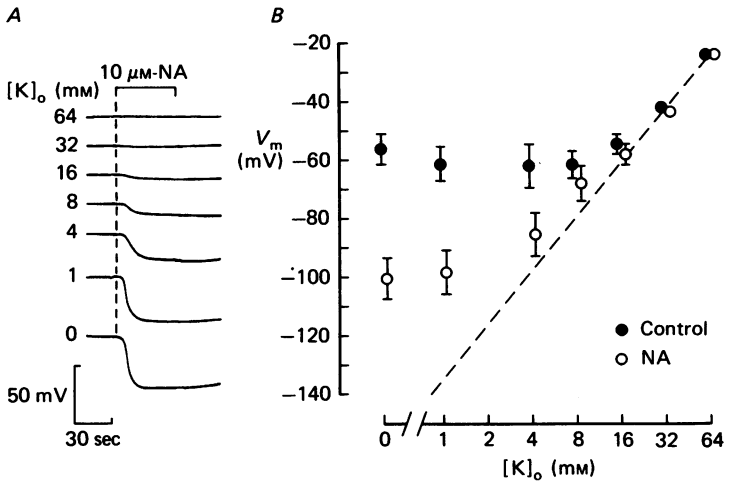

Fig. 4. $[K]_o$ dependence of the hyperpolarization caused by 10 μM-noradrenaline in low Cl solutions. *A*, sample chart recordings from a representative experiment, showing responses to 45 sec applications of noradrenaline in the presence of the K concentrations indicated beside each record. The dashed line marks the beginning of each application. *B*, graph summarizing responses to 10 μM-noradrenaline obtained from a total of seventeen preparations. The points give average values $\pm$S.D. of the membrane potentials measured just before (control), and after at least 40 sec of exposure to noradrenaline (NA), respectively. Each point is the average of from five to twenty measurements made on four to ten preparations except for the points at 32 mM extracellular K (four measurements, three preparations) and at 64 mM-extracellular K (three measurements, two preparations). The dashed line shows E_K calculated from the Nernst equation on the assumption that $[K]_i$ = 155 mM.

noradrenaline (10 μM) in a preparation exposed to a wide range of K concentrations. The hyperpolarization was negligibly small when $[K]_o$ was 64 mM, but its amplitude increased progressively as $[K]_o$ was lowered and reached 40–50 mV when $[K]_o$ was 1 mM or nominally zero. The results from this experiment, and from several similar experiments, are summarized in Fig. 4*B*, in which the average resting potentials at various $[K]_o$ levels, measured just before exposure to noradrenaline, are indicated by the filled circles, and the average membrane potentials recorded shortly after (40–120 sec) application of noradrenaline are represented by the open circles. The graph shows clearly that the amplitude of the noradrenaline-induced hyperpolarization increases relative to that obtained at 4 mM-extracellular K when $[K]_o$ is lowered, but decreases when $[K]_o$ is raised above 4 mM. It is important to notice that (just as in the case of the hyperpolarization caused by acetylcholine) this effect of $[K]_o$ is not due simply to the $[K]_o$-induced changes in membrane potential, since the control resting potential is approximately the same at $[K]_o$ levels of 1, 4 and 8 mM whereas

the sizes of the hyperpolarizations caused by the same dose of noradrenaline at those three $[K]_o$ levels are strikingly different.

Another feature shared by the noradrenaline- and acetylcholine-induced hyperpolarizations is that, at a fixed level of $[K]_o$, the amplitude of those hyperpolarizations becomes smaller as the extracellular Na concentration is reduced. This effect is

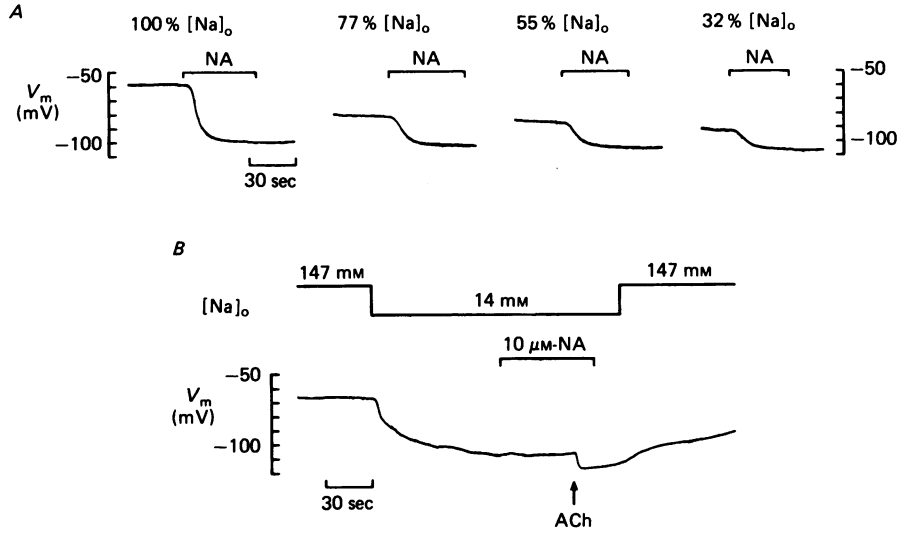

Fig. 5. $[Na]_o$ dependence of the hyperpolarization caused by 10 μM-noradrenaline. *A*, noradrenaline was applied as soon as a steady membrane potential had been reached in the low-Na solution; i.e. about 1 min after reducing $[Na]_o$ by substituting sucrose for Na isethionate. Between each test run the preparation was exposed to 100% Na solution for 15–20 min to allow complete recovery from the effects of noradrenaline and low $[Na]_o$. The records were obtained in the following chronological sequence: 100%, 32%, 55%, and 77% $[Na]_o$. The steady membrane potentials recorded in the presence of noradrenaline and in 100%, 77%, 55%, and 32% $[Na]_o$ were, respectively, −100·5, −102·5, −104·5, and −106 mV; however, membrane potential levels in 77%, 55%, and 32% $[Na]_o$ might be increased by up to 1, 3, and 5 mV, respectively, by corrections to take account of the liquid junction potentials expected to persist between the preparation and the remotely positioned reference electrode. 1 mM-K, low Cl solutions buffered with HCO_3/CO_2. *B*, after reducing $[Na]_o$ to about 10% by replacement with sucrose, 10 μM-noradrenaline caused practically no further hyperpolarization whereas a maximal concentration of ACh (drop of 10 mM-ACh directly into superfusion chamber) caused a further hyperpolarization of about 10 mV. All potential levels in 14 mM-Na should be increased by up to 7 mV to allow for the liquid junction potential. 1 mM-K, low Cl solutions buffered with HEPES.

illustrated in Fig. 5. The records in Fig. 5*A* show the hyperpolarizations caused by 40–45 sec applications of 10 μM-noradrenaline to a preparation exposed to 1 mM-K solution containing 100%, 77%, 55% or 32% of the normal level of $[Na]_o$ (Na isethionate being replaced isosmotically by sucrose). As previously described (Boyden *et al.* 1983), the resting potential increased towards the expected value of the K equilibrium potential, E_K, as $[Na]_o$ was lowered. Subsequent application of noradrenaline caused a further hyperpolarization towards E_K; the size of that hyperpolarization was smaller at lower levels of $[Na]_o$, although the final steady level of

the membrane potential in the presence of 10 μM-noradrenaline changed only slightly with changes in $[Na]_o$ (Fig. 5*A*).

At sufficiently low levels of $[Na]_o$, the noradrenaline-induced hyperpolarization became negligibly small, as illustrated by the changes in membrane potential shown in Fig. 5*B*. This record, obtained in another preparation exposed to 1 mM-$[K]_o$, shows that when the resting potential had been increased by lowering $[Na]_o$ to only 10% of its control level, application of 10 μM-noradrenaline caused essentially no further hyperpolarization. However, sudden application of a maximally effective dose of acetylcholine, still in the presence of low $[Na]_o$ and noradrenaline, caused a further rapid hyperpolarization of about 10 mV, towards the expected value of E_K. The same dose of acetylcholine caused a hyperpolarization of similar amplitude in low Na solution even in the absence of noradrenaline (not illustrated). In 100% $[Na]_o$, on the other hand, that dose of acetylcholine caused, in the same preparation, an average hyperpolarization of 54 mV amplitude (S.D. $\pm$8 mV, nine trials; cf. Fig. 4 of Boyden *et al.* 1983). Moreover, the membrane potential attained in the presence of that dose of acetylcholine was only slightly greater when $[Na]_o$ was 10% than when it was 100%.

The record in Fig. 5*B* serves also to confirm the result illustrated in Fig. 1*B*, that a maximally effective concentration of acetylcholine causes a greater hyperpolarization than that caused under the same conditions by a maximally effective concentration of noradrenaline.

Mechanism of the noradrenaline-induced hyperpolarization

So far, we have shown that noradrenaline causes, via activation of β-adrenoceptors, a dose-dependent hyperpolarization of cells of the coronary sinus, and that the size of the hyperpolarization caused by a maximally effective concentration of noradrenaline becomes smaller when the resting potential prior to application of the drug has been moved closer to E_K either by raising $[K]_o$ or by lowering $[Na]_o$. Since we know that the slope conductance of the membrane of coronary sinus cells increases as the membrane potential is increased toward E_K, possibly as a result of inwardly rectifying K conductance (Boyden *et al.* 1983), the results presented so far are compatible with two different kinds of mechanisms for the noradrenaline-induced hyperpolarization. Thus, we have already demonstrated that the membrane of resting coronary sinus cells is predominantly permeable to Na and K ions, and that an electrogenic Na/K pump makes a substantial contribution to the resting potential (Boyden *et al.* 1983), so that noradrenaline might either (*a*) increase the pump-mediated component of membrane potential, or (*b*) modify the permeability of the membrane to K and/or Na ions. The experiments reported below were designed to distinguish between these possibilities.

Effects of inhibiting the Na/K pump

The Na/K pump transports more Na ions out of the cell than it transports K ions into the cell, thereby continuously generating an outward (hyperpolarizing) current across the cell membrane (e.g. Thomas, 1972). If noradrenaline acts by increasing the size of that current, then inhibition of the Na/K pump would be expected to prevent, or at least markedly diminish, the noradrenaline-induced hyperpolarization. To test this suggestion, we investigated the response to application of noradrenaline after

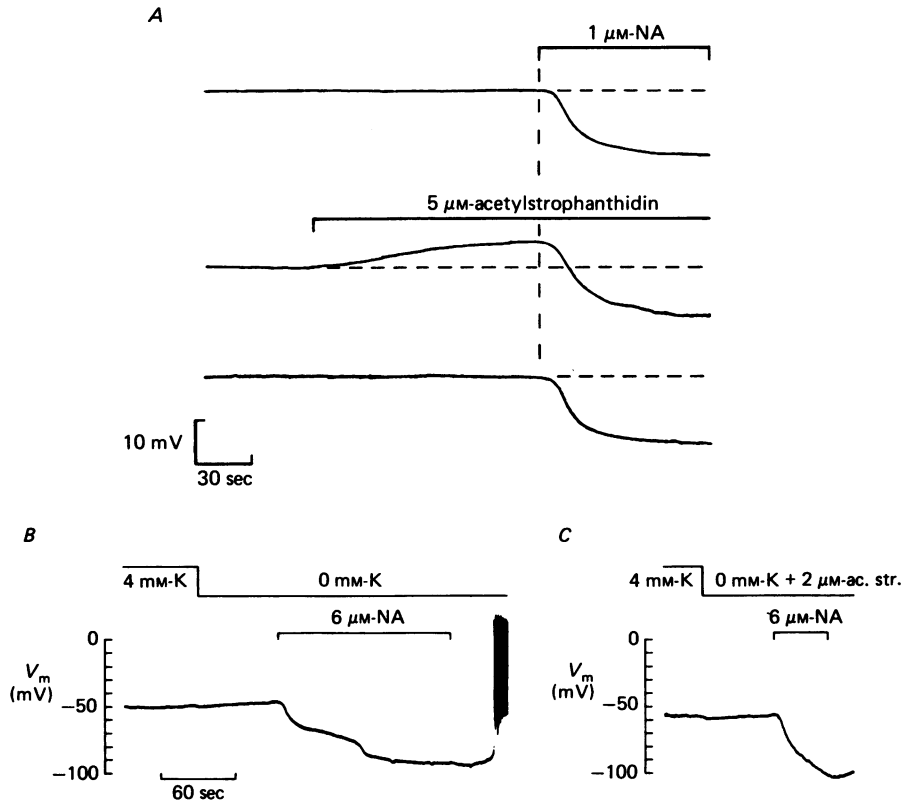

Fig. 6. The noradrenaline-induced hyperpolarization is not diminished by acetylstrophanthidin (ac. str.), and it persists in K-free solutions. *A*, three chart records of changes in membrane potential caused by application of 1 μM-noradrenaline (indicated by upper bar; start marked by vertical dashed line). 5 μM-acetylstrophanthidin was applied during the middle run only. The resting potentials, indicated by the horizontal dashed lines, for the three runs were −61, −61·5, and −63 mV, respectively. 4 mM-K, low Cl solution throughout. *B*, chart recording of changes in membrane potential in response to removal of extracellular K (indicated by upper line) and then application of 6 μM-noradrenaline (marked by bar). Noradrenaline causes a 50-mV hyperpolarization despite the nominal absence of extracellular K. The depolarization following removal of noradrenaline caused a burst of triggered activity. Low Cl solutions. *C*, the upper line shows the timing of the switch from 4 mM-K to K-free solution containing acetylstrophanthidin; the bar indicates the application of noradrenaline. Although the illustrated exposure to acetylstrophanthidin is brief, a prior $3\frac{3}{4}$ min exposure to K-free solution containing acetylstrophanthidin ended just 2 min before the start of this voltage record so that, presumably, some Na pump sites remained inhibited. Low-Cl solutions.

inhibiting the Na/K pump in two different ways. The first technique for inhibiting the pump was exposure of the preparations to micromolar concentrations of the cardiac steroid acetylstrophanthidin, as illustrated in Fig. 6*A*. The upper record shows the hyperpolarization of 14·5 mV caused by application of 1 μM-noradrenaline under control conditions. The noradrenaline was then washed out for about 5 min to allow the membrane potential to return to its steady resting level. The middle record shows that exposure to 5 μM-acetylstrophanthidin then resulted in a depolariza-

tion which reached an approximately steady amplitude of 6 mV after 2 min; that depolarization, presumably, largely reflects abolition of the steady-state Na/K pump current (see Boyden *et al.* 1983). Application of the same dose of noradrenaline in the maintained presence of acetylstrophanthidin then caused a 17 mV hyperpolarization. The lower record was obtained after washing out both acetylstrophanthidin and noradrenaline for 25 min, and shows that the hyperpolarization induced by re-exposure to noradrenaline had regained the control amplitude of 14·5 mV. In all three records the time courses of the noradrenaline-induced hyperpolarizations are seen to be practically identical. Similar results were obtained in other experiments in which a range of noradrenaline concentrations (0·1–10 μM) was tested in the absence and presence of acetylstrophanthidin (5–10 μM). These results show that inhibition of the Na/K pump by acetylstrophanthidin neither abolishes nor diminishes the noradrenaline-induced hyperpolarization.

Since the Na/K pump requires extracellular K ions for its operation, the second way we reduced pump activity was by superfusing preparations with K-free solution. In the record shown in Fig. 6*B*, sudden reduction of $[K]_o$ from 4 mM to 0 was followed by a small, slow depolarization of a few millivolts. Subsequent application of 6 μM-noradrenaline caused a marked hyperpolarization with a rather complex time course: within 2 min of applying noradrenaline the amplitude of the hyperpolarization was 48 mV. Shortly after washing out the noradrenaline, while K-free superfusion was maintained, the membrane potential began to decline, at first slowly and then abruptly. That abrupt depolarization gave rise to a burst of triggered action potentials (see Wit & Cranefield, 1977; Wit *et al.* 1981). The phase of abrupt depolarization occurred over about the same voltage range as did the rapid phase of hyperpolarization seen earlier in the same record, after 1 min of exposure to noradrenaline, and it seems likely that both arose because the slope conductance of the membrane was very small or even negative, over that region of the steady-state current–voltage relationship (see Boyden *et al.* 1983). The record in Fig. 6*C* shows that, even in the presence of both K-free solution and acetylstrophanthidin, application of 6 μM-noradrenaline still caused a marked hyperpolarization, almost 50 mV in amplitude. Although the preparation had been exposed to acetylstrophanthidin for only 60 sec when noradrenaline was applied, at the end of the exposure to noradrenaline, acetylstrophanthidin had been present for 100 sec, long enough to abolish completely any pump activity persisting in the presence of K-free solution (Fig. 6*A*). Taken together, these results strongly suggest that the noradrenaline-induced hyperpolarization does not result from an increase in the Na/K pump current. That being the case, the most likely explanation for the hyperpolarization is that it is due to a change in the permeability of the cell membrane to Na and/or K ions. We therefore carried out experiments to see if we could detect any changes in membrane conductance associated with the action of noradrenaline (cf. Dudel & Trautwein, 1956).

Experiments to determine changes in membrane conductance

For these experiments to measure conductance, we dissected very small preparations from the coronary sinus, using fine iris scissors. The average ($\pm$S.D.) length, and width, of these preparations was 1·5$\pm$0·7 mm, and 0·25$\pm$0·05 mm (n = 16), respectively.

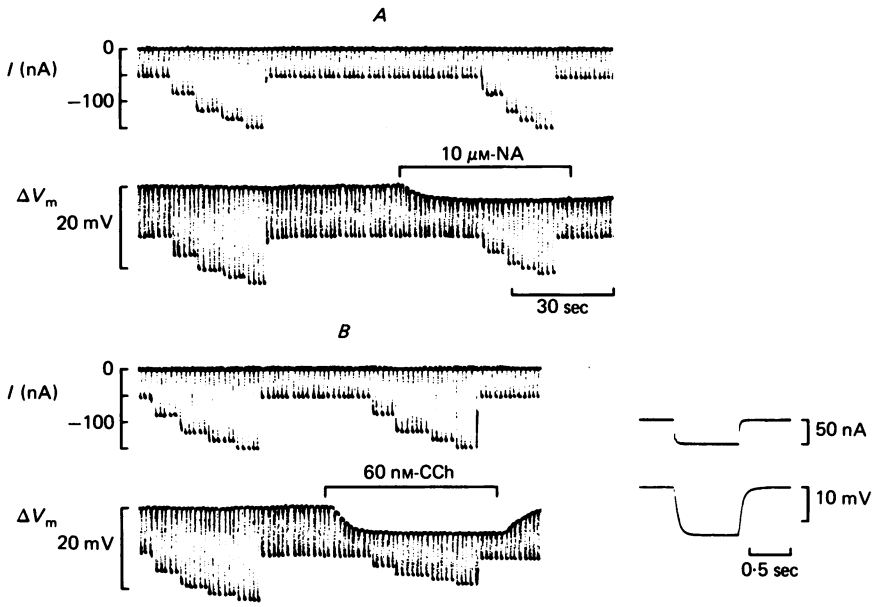

Fig. 7. Changes in membrane conductance caused by noradrenaline and carbachol in a small coronary sinus preparation exposed to 16 mM-K, low-Cl solution. In both *A* and *B*, the upper traces show the 800 ms hyperpolarizing current pulses injected via a micro-electrode at the mid-point of the preparation, and the lower traces show the resulting changes in membrane potential recorded nearby via a second micro-electrode. *Inset*, chart recordings at a faster paper speed to show that the membrane potential reached an approximately steady level during each current pulse. The same stepwise increases in current amplitude were repeated in the absence and in the presence of each agonist (indicated by the bars). In this experiment, and in some others involving $[K]_o$ levels of 16 mM or higher, 2 μM-verapamil was added to the high-K solutions to diminish the excitability of the depolarized coronary sinus cells and thereby facilitate completion of the experimental protocol.

Each preparation was impaled with two micro-electrodes, a current-injecting micro-electrode near the centre of the preparation and a voltage-recording micro-electrode 0·1–0·3 mm away from it. Rectangular hyperpolarizing current pulses of low amplitude and 0·5 to 1·0 sec duration were injected at regular intervals of 3–6 sec. The duration of the pulses was sufficient to allow the membrane potential to reach a new steady level during each pulse (see inset to Fig. 7), and their amplitude was increased in a stepwise manner so that the relationship between steady membrane potential and applied current could be determined over a suitably wide range. Since the hyperpolarization caused by the muscarinic cholinergic agonists acetylcholine and carbachol had several characteristics in common with the noradrenaline-induced hyperpolarization and since the mechanism of the cholinergic effect is known, we compared, in a series of experiments, the changes in conductance caused by noradrenaline with those caused by carbachol. Sample results from a typical experiment, carried out in 16 mM-K, low-Cl solution, are illustrated in Fig. 7.

The upper records in Figs. 7*A* and 7*B* show the amplitudes of the injected current pulses, which were progressively increased from −50 to −150 nA both in the absence

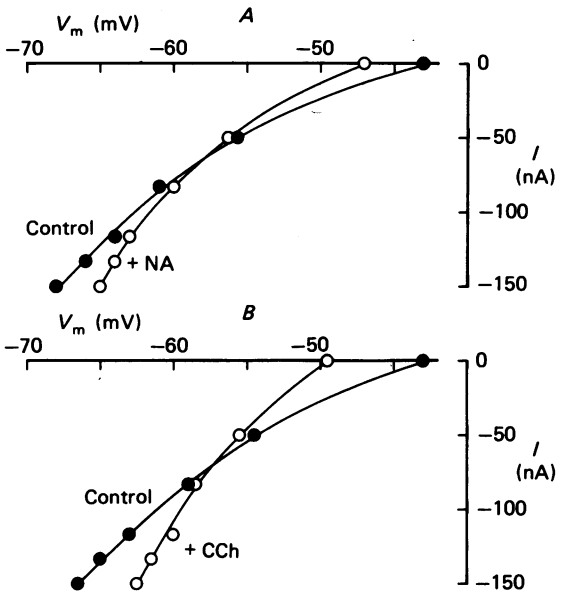

Fig. 8. Current–voltage curves plotted from the data of Fig. 7. In both (*A*) and (*B*), the abscissa gives the steady level of membrane potential reached during the injection of current pulses of amplitude given on the ordinate. The filled circles show control results obtained immediately before drug application, and the open circles show results obtained a few seconds after application of 10 μM-noradrenaline (*A*), or of 60 nM-carbachol (*B*), respectively. The curves were drawn through the points by eye. Note that the 'steady-state' current–voltage relationships determined in the presence and absence of each drug cross each other at approximately the same potential, about −58 mV in this case.

and in the presence of noradrenaline and carbachol. The lower records in Figs. 7*A* and 7*B* show the resulting changes in membrane potential. The zero-current potential, or resting potential, was increased by about 4 mV during the 100 sec exposure to 10 μM-noradrenaline (Fig. 7*A*), and was increased by almost 7 mV during exposure to 60 nM-carbachol (Fig. 7*B*). Each drug caused a reduction in the size of the voltage deflexions elicited by each amplitude of current pulse. Equally important, however, is the finding that the level of membrane potential reached during application of the largest current pulses was actually more *positive* in the presence of either noradrenaline (Fig. 7*A*) or carbachol (Fig. 7*B*). This effect is illustrated more clearly by the steady-state current–voltage relationships presented in Fig. 8. These were plotted directly from the data of Fig. 7: the abscissae give the steady levels of membrane potential reached during the current pulses of amplitudes given by the ordinates, in the presence of noradrenaline (open circles, Fig. 8*A*) or carbachol (open circles, Fig. 8*B*), or in the absence of either agent (filled circles, Figs. 8*A* and 8*B*). The arbitrary curves were fitted to the points by eye, and they make it clear that the current–voltage relationships obtained in the presence of either noradrenaline or carbachol cross the control current–voltage curves obtained in their absence; moreover they do so at approximately the same potential which, in this instance, was about −58 mV. In addition, at all voltages illustrated, the current–voltage curves

obtained in the presence of these agents have steeper slopes (greater slope conductances) than the control curves.

The crossover of the current–voltage curves shown in Fig. 8 reflects the finding that the level of membrane potential reached with large hyperpolarizing currents became more positive during exposure to noradrenaline or carbachol. These results imply that

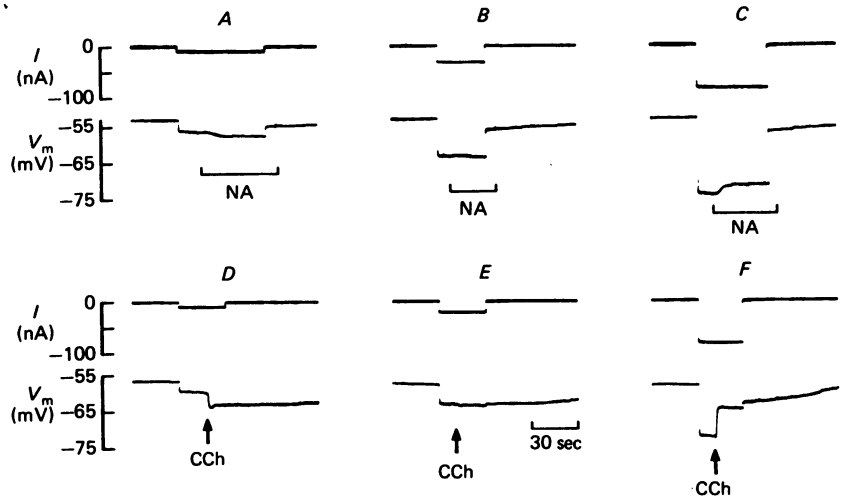

Fig. 9. Direct demonstration of similar reversal potentials for the effects of 10 μM-noradrenaline (*A*, *B*, *C*) and of carbachol (*D*, *E*, *F*). The 16 mM-K, low-Cl solutions contained 1 μM-verapamil; maximally effective doses of carbachol were applied as drops of 10 mM-carbachol solution introduced into the superfusate just upstream from the preparation. The upper traces show the prolonged, hyperpolarizing current pulses injected via the second intracellular micro-electrode. The lower, membrane potential, traces show that noradrenaline and carbachol both cause *depolarization* during injection of strongly hyperpolarizing currents (*C*, *F*), both have little effect during moderate hyperpolarization (*B*, *E*), and both cause *hyperpolarization* during injection of weakly hyperpolarizing currents (*A*, *D*). Comparison of the voltage steps at the beginning and end of each current pulse (i.e. in the absence and in the presence of the drug) shows that maximal activation of the muscarinic cholinergic receptors (*D*, *E*, *F*) causes a much greater increase in membrane slope conductance than does maximal activation of the β-adrenoceptors (*A*, *B*, *C*).

application of either noradrenaline or carbachol *during* the maintained injection of a sufficiently large hyperpolarizing current should lead to a drug-induced *depolarization* instead of a hyperpolarization. The results shown in Fig. 9 verify this prediction. The top half of the Figure shows the changes in membrane potential (lower records) caused by brief exposures to 10 μM-noradrenaline in a preparation hyperpolarized to different levels of potential by inward currents (shown in the upper records) of progressively increasing amplitude (Fig. 9*A*, *B*, *C*). The results in the bottom half of the figure were obtained a few minutes later in the same preparation, and show similar changes in membrane potential in response to sudden application of carbachol during prolonged hyperpolarizing current pulses of increasing magnitudes (Fig. 9*D*, *E*, *F*). When only weak (5 nA) currents were injected, bringing the membrane potential to about −57 mV, application of either noradrenaline (Fig. 9*A*) or

carbachol (Fig. 9*D*) caused a distinct hyperpolarization. During injection of larger hyperpolarizing currents, which brought the membrane potential to about −63 mV, neither agent caused any obvious change in potential (Fig. 9*B*, *E*). In contrast, during application of even larger steady currents which hyperpolarized the preparation to about −73 mV, the addition of either noradrenaline (Fig. 9*C*) or carbachol (Fig. 9*F*) clearly caused *depolarization*. The level of steady membrane potential at which these drugs caused neither hyperpolarization nor depolarization in this preparation (Fig. 9) is similar to the level of potential at which the current–voltage curves, determined in the presence and in the absence of the drugs, crossed each other (Fig. 8); those

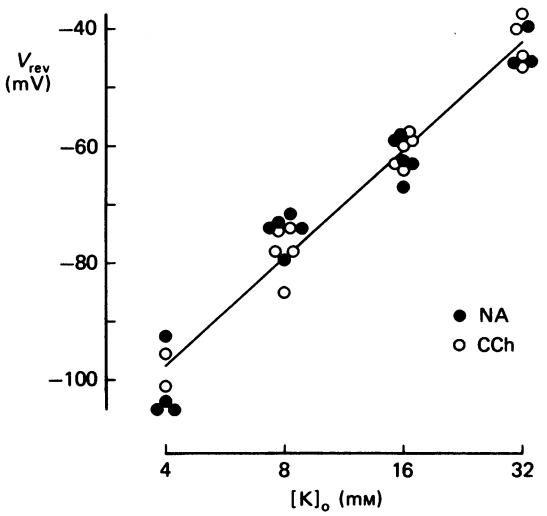

Fig. 10. Semilogarithmic plot comparing the $[K]_o$ dependence of the reversal potentials (V_{rev}) for the effects of noradrenaline (filled circles; concentration, 10 μM) and of carbachol (open circles; concentration 30–80 nM). Results from ten preparations are summarized; each point represents a single determination of the crossover potential of steady-state current–voltage curves obtained in the presence and in the absence of the drugs, as shown in Fig. 8. At $[K]_o$ levels of 4, 8, 16 and 32 mM, results were obtained from two, four, three, and three preparations, respectively. Most experiments yielded results at only one $[K]_o$ level, but two preparations were studied at both 8 mM and 32 mM-K. Low Cl solutions were used for all these experiments. The straight line shows E_K calculated from the Nernst equation for $[K]_i$ = 155 mM.

current–voltage curves were obtained in a different preparation, but under otherwise similar experimental conditions (16 mM-K, low chloride solution).

The very fact that noradrenaline can cause depolarization of preparations hyperpolarized by injection of a steady current (Fig. 8*C*) makes it extremely unlikely that noradrenaline acts solely by reducing the permeability of the cell membrane to Na ions. The reason is that at all negative values of membrane potential, the driving force on Na ions crossing the membrane is inward, so that any reduction in Na permeability must always result in a reduction in inward (depolarizing) Na current, a reduction which must always cause hyperpolarization. Equally, the fact that the 'crossover', or 'reversal', potential for the effect of noradrenaline lies close to the expected value

of E_K (−61 mV for 16 mM-K, assuming that $[K]_i$ is 155 mM), strongly suggests that noradrenaline acts by increasing the permeability of the cell membrane to K ions, because such a permeability increase would enhance inward K current at potentials negative to E_K, would cause no change in current at E_K, and would enhance outward K current at potentials positive to E_K. The close similarity of the reversal potentials for the effects of noradrenaline and of carbachol in Figs. 8 and 9 provides some support for this conclusion since muscarinic cholinergic agonists are well known to increase K permeability in other atrial cells (Hutter, 1964).

If, indeed, noradrenaline does increase K permeability in coronary sinus cells, then the reversal potential for the effect of noradrenaline must be expected to vary with changes in $[K]_o$ just as E_K does (as predicted by the Nernst equation). The graph in Fig. 10 summarizes results obtained from ten coronary sinus preparations and shows, for four different levels of $[K]_o$, individual values for the reversal potentials (V_{rev}) for the effects of noradrenaline (filled circles) and of carbachol (open circles). The reversal potentials were determined as the crossover points of current–voltage curves obtained with and without the drugs, as illustrated in Fig. 8. The straight line, which provides a reasonably good fit to the experimental points, shows E_K calculated on the assumption that $[K]_i$ is 155 mM. We can conclude from these experiments, then, that both noradrenaline and carbachol increase the specific K permeability of the resting membrane of coronary sinus cells, although their similar actions are mediated via pharmacologically distinct receptors.

DISCUSSION

Our results show that noradrenaline causes a dose-dependent hyperpolarization of cells of the canine coronary sinus similar to the hyperpolarization of canine atrial trabeculae reported by Dudel & Trautwein (1956), that this effect of noradrenaline is mediated via activation of β-adrenoceptors, and that the effect is not due to enhanced activity of an electrogenic Na/K pump but is caused, rather, by an increase in membrane K permeability. The latter conclusion is striking, since we find that each of the classically antagonistic autonomic neurotransmitters, noradrenaline and acetylcholine, acts on coronary sinus cells to increase membrane permeability to K ions.

The dose–response plot in Fig. 2 requires some comment. Although the mean normalized hyperpolarizations can be fitted reasonably well by a simple saturable binding curve, that should not be taken as evidence that the amplitude of the noradrenaline-induced hyperpolarization is directly proportional to the fraction of β-adrenoceptors occupied by noradrenaline. Thus, the results presented in subsequent Figures indicate that noradrenaline acts by increasing membrane K permeability, i.e. membrane K conductance (see Figs. 6–10), so that the hyperpolarizing current generated by a given increment in K conductance may be expected to depend on the electrochemical driving force for movement of K ions across the membrane: that driving force declines as the membrane potential approaches E_K. In other words, the size of the noradrenaline-induced hyperpolarization is not expected to be directly proportional to the noradrenaline-induced increment in membrane conductance. In theory, it would be possible to make allowance for this effect of 'non-linear

summation' (Martin, 1955; cf. Glitsch & Pott, 1978) of the hyperpolarizations caused by identical, noradrenaline-induced, increments in K conductance if (*a*) the underlying increments in K conductance are small relative to the resting membrane conductance, (*b*) the noradrenaline-induced conductance is linear (i.e. voltage-independent), and (*c*) the resting membrane conductance is linear. Although we do not at present have detailed information concerning the voltage dependence of the noradrenaline-induced conductance, an assumption of linearity would probably be a reasonable first approximation if the maximum amplitude of the hyperpolarizations were small. This was certainly not the case, however, an average hyperpolarization of 23 mV being caused by 10 μM-noradrenaline in the experiments summarized in Fig. 2. Moreover, those hyperpolarizations occurred over a voltage range in which the resting membrane conductance is demonstrably non-linear (e.g. Fig. 1*C*; see also Boyden *et al.* 1983). Quantitative correction of the dose–response data in Fig. 2 for the effect of non-linear summation would, therefore, seem to be unprofitable. On the other hand, it can be argued that, qualitatively, correction for the effects of non-linear summation would tend to augment the responses to the highest concentrations, while correction for the known shape of the steady-state current–voltage relationship would probably tend to diminish mainly the responses to the intermediate concentrations of noradrenaline. The over-all effect would be a tendency for the corrected dose–response curve to be shifted slightly to the right. Regardless of such corrections, however, it seems safe to conclude that the foot of the 'true' dose–response curve (in terms of noradrenaline-induced K conductance) occurs at about 10 nM-noradrenaline and that the top of that dose–response curve occurs near 10 μM.

In view of the above-mentioned uncertainties, it does not seem worthwhile to discuss the dose–response curve in any detail except, perhaps, to note that the apparent affinity of the receptors mediating the noradrenaline-induced increase in K conductance in coronary sinus cells seems more comparable to values previously reported for the effects of adrenaline and noradrenaline on processes related to the secondary inward (Ca) current (e.g. Carmeliet & Vereecke, 1969; Vassort, Rougier, Garnier, Sauviat, Coraboeuf & Gargouil, 1969; Reuter, 1974; Pappano & Carmeliet, 1979; Kass & Wiegers, 1982; cf. Grabowski, Luttgau & Schultze, 1978) than to values reported for effects on processes related to the Purkinje fibre pace-maker current (Tsien, 1974; cf. Rosen, Hordef, Ilvento & Danilo, 1977; Grabowski *et al.* 1978).

Exposure to catecholamines has been shown to increase the resting potential of cells in various tissues, for example, skeletal muscle (see, e.g. Clausen & Flatman, 1977, for references), and smooth muscle (for review, see Bulbring, Ohashi & Tomita, 1981), as well as cardiac muscle (e.g. Dudel & Trautwein, 1956; Trautwein & Schmidt, 1960; Hoffman & Singer, 1967; Wit & Cranefield, 1977; Gelband, Rosen, Myerburg, Bush, Bassett & Hoffman, 1977). In some instances, the catecholamine-induced hyperpolarization has been shown to be diminished following prolonged exposure to cardiac steroids such as ouabain (e.g. Clausen & Flatman, 1977) or to metabolic inhibitors (e.g. Trautwein & Schmidt, 1960), and it has therefore been suggested that the catecholamines act by enhancing electrogenic Na/K pump activity. It is clear that this cannot be the explanation for the catecholamine-induced hyperpolarization of cells in the canine coronary sinus reported here and elsewhere (see, e.g. Fig. 6 of Wit, Cranefield & Gadsby, 1980; Wit & Cranefield, 1977) because that hyperpolarization

is not diminished by treatment with 5 μM-acetylstrophanthidin (Fig. 6*A*, above), a concentration sufficient to completely inhibit the electrogenic Na/K pump of canine coronary sinus cells (cf. Fig. 7 of Boyden *et al.* 1983; Wit *et al.* 1981), and of canine cardiac Purkinje cells (Gadsby & Cranefield, 1979*a*, *b*). Moreover, a large catecholamine-induced hyperpolarization can be elicited in coronary sinus cells after the Na/K pump has been inhibited by exposure either to K-free fluid, or to K-free fluid containing acetylstrophanthidin (Fig. 6*B*, *C*, above). Thus the rapid hyperpolarization that develops within the first minute or two of exposing coronary sinus cells to β-adrenergic catecholamines is most readily attributed to an increase in membrane K conductance.

We have recently reported a similar hyperpolarizing effect of the β-adrenergic catecholamine, isoproterenol, on canine cardiac Purkinje fibres: a voltage-clamp investigation of the isoproterenol-induced current showed that it was not diminished by exposing the fibres either to 5 μM-acetylstrophanthidin or to K-free solution (Gadsby & Cranefield, 1981), and revealed that, at extracellular K concentrations of 4, 8 and 16 mM the current reversed direction at voltages close to calculated levels of E_K (Cranefield & Gadsby, 1981). We conclude therefore that, at least in the canine heart, β-adrenergic catecholamines increase membrane K permeability of resting cells in the coronary sinus, and in Purkinje fibres, and, probably, also in atrial myocardium, because Dudel & Trautwein (1956) demonstrated an increase in the slope conductance of canine atrial trabeculae during the hyperpolarization caused by exposure to high doses of adrenaline.

We would emphasize that our experiments do not rule out the possibility that catecholamines may cause indirectly some small increase in Na/K pump rate, for example, via modification of cellular metabolic processes. However, a more likely mechanism for a transient, indirect stimulation of the Na/K pump during exposure of some kinds of preparations to catecholamines might be via a temporary accumulation of K in restricted extracellular spaces secondary to a catecholamine-induced increase in K conductance; the raised extracellular K concentration then causing an increase in pump rate. Such an effect might provide an explanation for reported observations (mentioned above) of a diminution of the catecholamine-induced hyperpolarization caused by exposure to a cardiac steroid. Of course, prolonged exposure to pump inhibitors must be expected to cause E_K to become less negative, and if the resting potential were to decline less than E_K declines, a reduction in the size of the catecholamine-induced hyperpolarization (with respect to control) would be expected even if the catecholamine acted solely to increase membrane K conductance. It is clear that if catecholamines were to act by increasing the sensitivity of the Na/K pump to $[K]_o$ or to $[Na]_i$, or by increasing the density of functional pump sites, then an increase in pump current, and hence membrane hyperpolarization, could occur only at the expense of an increased rate of Na extrusion. Unless Na influx were simultaneously increased, $[Na]_i$ would fall and, since $[Na]_i$ determines the rate of Na efflux, both would decline towards a new steady level at which Na influx and Na efflux were again in balance. In other words, the rate of Na extrusion would be increased only *transiently*, decaying with the time course of the fall in $[Na]_i$, i.e. with a half-time of a minute or two (cf. Fig. 7 of Boyden *et al.* 1983). A report of an adrenaline-induced increase in the sensitivity of the Na/K pump to $[K]_o$ was made recently by Akasu, Ohta & Koketsu (1978). They studied bullfrog atria under

sucrose-gap voltage clamp, and measured the current generated by the Na/K pump during one-minute exposures to various $[K]_o$ levels following prolonged exposure to K-free fluid. Unfortunately, their paper does not include all of the detailed experimental information needed (e.g. no values are given for the holding potentials) to assess any possible contribution made to their results by extracellular K accumulation secondary to an adrenaline-induced increase in K conductance. In general, there is only one straightforward way in which catecholamines could cause a *maintained* increase in pump current without a concomitant increase in the rate of Na extrusion, hence, without any change in $[Na]_i$, and that would be by increasing the stoichiometric ratio of Na/K exchange. Although a precise estimate of the Na/K coupling ratio of the pump is not available for most cell types (other than red blood cells; Thomas, 1972), there is reasonable evidence, at least in some cell types, that the coupling ratio remains approximately constant in spite of relatively large changes in pump rate elicited by alterations of the concentrations of both extracellular and intracellular activator cations over a fairly wide concentration range (Thomas, 1969; Gadsby, 1980; Eisner & Lederer, 1980; Eisner, Lederer & Vaughan-Jones, 1981*a*, *b*). In view of this, a convincing demonstration that β-catecholamines can increase the Na/K coupling ratio in any cell type would be of considerable interest; we are not aware of any such demonstration.

Our conclusion that noradrenaline hyperpolarizes coronary sinus cells by increasing the permeability of the membrane to K ions raises three additional questions:

(1) Can we rule out the possibility that noradrenaline causes not only an increase in the K permeability (P_K), but also a decrease in the Na permeability (P_{Na}), of the cell membrane? As already mentioned, a reduction in P_{Na} is expected to diminish inward current at all negative membrane potentials, whereas an increase in P_K is expected to increase inward current negative to E_K but to increase outward current positive to E_K. The net effect of a combined decrease in P_{Na} and increase in P_K would be to shift, in the negative direction, the potential at which the total noradrenaline-induced current is zero, i.e. the crossover potential. The extent of that shift would depend on the relative amount by which each permeability was altered and on the voltage dependence of the changes in permeability, and so would be likely to vary with $[K]_o$. Therefore, if noradrenaline-induced changes in P_{Na} were at all substantial, the crossover potentials would be unlikely to vary with changes in $[K]_o$ simply according to the predictions of the Nernst equation. In spite of the scatter of the data in Fig. 10, their agreement with both the position and the slope of the line that indicates E_K for a $[K]_i$ of 155 mM suggests that, as far as we can tell, noradrenaline causes a specific increase in the permeability of the membrane to K ions.

(2) Since both noradrenaline and acetylcholine increase the K permeability, why is the hyperpolarization induced by a maximally effective concentration of acetylcholine always greater than that induced by a maximal concentration of noradrenaline? Presumably, the maximum K conductance that can be induced by acetylcholine is much larger than that inducible by noradrenaline as indicated by the records of Fig. 9, for example, in which the voltage steps on termination of the current pulses are seen to be much smaller in the presence of a maximal concentration of carbachol than in the presence of a maximal concentration of noradrenaline. During exposure to a high concentration of acetylcholine or carbachol, the resting potential of

coronary sinus cells lies close to the expected value of E_K at all levels of $[K]_o$ from 1 to 150 mM (Boyden *et al.* 1982), suggesting that the permeability of the cell membrane to K ions is then much greater than its permeability to other ions such as Na, Ca or Mg ions. The hyperpolarization caused by a high concentration of noradrenaline, on the other hand, leaves the membrane potential still far from E_K at most levels of $[K]_o$ (Fig. 4*B*); moreover, application of a high dose of acetylcholine then causes a substantial further hyperpolarization to a level close to E_K (e.g. Fig. 5*B*). This suggests that even when the noradrenaline-induced K permeability is maximal, the permeability of the membrane to other ions still makes an important contribution to the membrane potential. Indeed, a reasonable fit to the membrane potentials recorded in noradrenaline at various $[K]_o$ levels (open circles, Fig. 4*B*) can be obtained by using the Goldman (1943), Hodgkin & Katz (1949) equation with $\alpha = P_{Na}/P_K = 0{\cdot}02$, $[K]_o + [Na]_o = 155$ mM, $[K]_i = 155$ mM, $[Na]_i = 10$ mM; the control data in Fig. 4*B* (filled circles) can be fitted by the same equation but with a larger α, 0·07, whereas the membrane potentials recorded in the presence of acetylcholine (Fig. 4 of Boyden *et al.* 1983) require a much smaller α, of about 0·005.

(3) Since β-adrenergic catecholamine action is well known to modulate transmembrane Ca movement, at least in beating heart cells, is it likely that the noradrenaline-induced K conductance is Ca-dependent, i.e. does it arise secondary to an increase in the intracellular Ca concentration, $[Ca]_i$? Although the existence of a steady-state component of Ca current is reasonably well established for some cardiac cells (Gibbons & Fozzard, 1975; Kass, Siegelbaum & Tsien, 1976), and although it is possible that such a component in coronary sinus cells might be augmented by the action of noradrenaline, it seems unlikely that any such increase in resting Ca influx would occur at voltages negative to about −40 mV, the apparent threshold for activation of Ca current (see, e.g. Reuter, 1973). Figs. 4 and 5 (above), however, demonstrate clearly that noradrenaline causes marked hyperpolarization of cells with initial resting potentials more negative than that threshold by at least 30 to 40 mV. Furthermore, the record shown in Fig. 9*C* indicates that the noradrenaline-induced increase in K conductance can arise in a preparation hyperpolarized to a steady potential of −73 mV. In addition, on no occasion was the noradrenaline-induced hyperpolarization seen to be preceded by any detectable depolarization, so that any increase in Ca influx postulated to raise $[Ca]_i$ and hence to activate the K conductance must be supposed to occur by an electrically silent means. Of course, our experiments do not allow us to rule out the possibility that noradrenaline might cause $[Ca]_i$ to rise in some other way (cf. Niedergerke & Page, 1981) and thereby enhance Ca-activated K conductance.

Whatever the molecular mechanism turns out to be, the results presented here make it clear that the β-catecholamine-induced hyperpolarization of coronary sinus cells results not from enhanced activity of the Na/K pump but from an increase in the permeability of the cell membrane to K ions.

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