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β_2-ADRENOCEPTORS MEDIATE THE STIMULATING EFFECT OF ADRENALINE ON ACTIVE ELECTROGENIC NA-K-TRANSPORT IN RAT SOLEUS MUSCLE

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1 The relative role of β_1- and β_2-adrenoceptors in mediating the stimulating effect of adrenaline on active electrogenic Na-K-transport has been assessed in experiments on rat soleus muscles *in vitro* and *in vivo*.

2 In the rat isolated soleus muscle, adrenaline (10^{-6} M) increases the resting membrane potential (E_M) by 5.8 mV and stimulates ^{22}Na-efflux and ouabain-suppressible ^{42}K-uptake by 91 and 94%, respectively.

3 All of these effects are completely blocked by propranolol (10^{-5} M), whereas the β_1-selective adrenoceptor antagonist, metoprolol, was found to be at least 50 times less potent.

4 The β_2-adrenoceptor agonist, salbutamol, was at least 100 times as potent as H133/22 (a β_1-selective agonist) in stimulating ^{22}Na-efflux and ^{42}K-influx.

5 In experiments performed under pentobarbitone anaesthesia, the intravenous injection of adrenaline (5 μg) or salbutamol (0.5 to 50 μg) led to a rapid and marked increase in the E_M of the exposed soleus muscle. This hyperpolarizing effect could not be accounted for by the concomitant, relatively modest change in extracellular K.

Introduction

It is well documented that catecholamines stimulate active Na-K-transport and augment the resting membrane potential (E_M) in isolated skeletal muscle (Dockry, Kernan & Tangney, 1966; Evans & Smith, 1973; Tashiro, 1973; Hays, Dwyer, Horowicz & Swift, 1974; for reviews, see Bowman & Nott, 1969; Daniel, Paton, Taylor & Hodgson, 1970; Bowman, 1979). In a combined electrophysiological and ionic flux study, we have shown that in the isolated soleus muscle of the rat, catecholamines at concentrations down to the physiological level induce a hyperpolarization, which is likely to be related to a stimulation of active coupled Na-K-transport (Clausen & Flatman, 1977). We concluded that these effects were mediated by β-adrenoceptors and were possibly the outcome of stimulation of adenylate cyclase.

In the present study, the effects of adrenaline on Na-K-transport and E_M have been examined in more detail by use of β_1- and β_2-selective agonists and antagonists. Furthermore, as in the intact organism, catecholamines induce hypokalaemia (Dury, 1951; Leitch, Clancy, Costello & Flenley, 1976), and may be used in the treatment of hyperkalaemia (Wang & Clausen, 1976), it was of interest to determine the effects of catecholamines on E_M *in vivo* (see Kendig & Bunker, 1972). Some of the results described in this paper have been presented in a preliminary communication (Flatman & Clausen, 1978).

Methods

All experiments were performed on fed female or male rats of the Wistar strain. For measurements of Na-K-transport, soleus muscles were obtained from rats weighing 60 to 70 g. Some membrane potential (E_M) recordings were made with muscles from rats of this size to allow comparison with the flux measurements. However, since more stable E_M recordings could be made in larger muscles, most of the E_M studies were performed on muscles from animals in the weight range 200 to 400 g. We have previously shown that the E_M response to adrenoceptor stimulants is similar in large and small muscles (Clausen & Flatman, 1977).

In vitro *experiments*

The measurements of membrane potentials and isotopic fluxes of Na and K were carried out as de-

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scribed previously (Clausen & Flatman, 1977; Clausen & Kohn, 1977). Soleus muscles were dissected out, washed with saline at room temperature and equilibrated in Krebs-Ringer bicarbonate buffer which was continuously gassed with a mixture of 95% O_2 and 5% CO_2. The incubation medium had the following composition (mM): NaCl 120.2, $NaHCO_3$ 25.1, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.27. For the measurement of membrane potentials, the muscles were mounted in thermostatically controlled (30°C) open perfused chambers, to allow vertical impalement of individual fibres under a stereomicroscope. Usually, serial penetrations were made across the muscles, and in order to allow a more detailed characterization of the time course of action, the microelectrode was often left in a cell whilst the drug under consideration was added to the bath. When the membrane potential had reached a new steady state, the electrode was withdrawn, and a new series of impalements were performed. Figure 1 shows an example of recordings obtained in an experiment with salbutamol. For the measurement of ^{22}Na-efflux, muscles were loaded for 60 min in buffer containing ^{22}Na, 10 μCi/ml. They were then washed in a series of tubes containing non-radioactive medium to which the drug under study was added at a moment when the washout of ^{22}Na could be ascertained to reflect efflux of sodium from the intracellular compartment. The fractional loss of isotopic Na was determined, and for each compound tested, the values obtained within the first 10 min of exposure were used. ^{42}K-uptake was determined by incubating muscles for 20 min in buffer containing ^{42}K without or with ouabain (10^{-3} M). From the amount of ^{42}K accumulated, the ouabain-suppressible component of ^{42}K-influx could be calculated and the effect of drugs added to the ^{42}K-containing medium assessed.

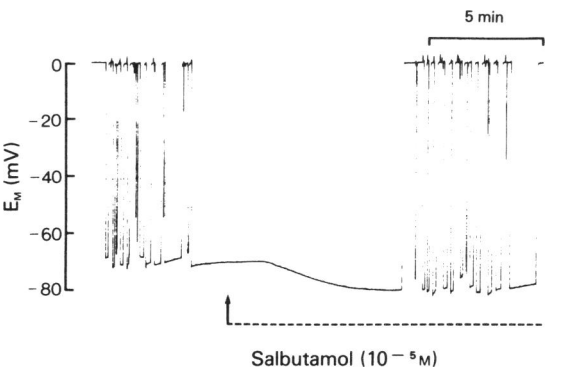

Figure 1 The action of salbutamol (10^{-5} M) on the E_M of a soleus muscle of a 100 g rat. A flat-bed recording of E_M measurements of superficial muscle fibres. A series of muscle fibre penetrations was first performed (E_M -70.6 ± 0.6 mV; 10 fibres). The criteria of acceptance for muscle fibre penetrations have been given in Clausen & Flatman (1977). In some fibres the electrode resistance rose on penetration but on slight withdrawal of the electrode its resistance returned to values differing less than 10% from the pre-penetration level. On other occasions the penetration was highly unstable and the electrode came out of the cell before the E_M could be accurately measured.

The electrode was then allowed to remain in a fibre and the muscle was rapidly exposed to a bath concentration of 10^{-5} M salbutamol. After a short latent period the E_M started to increase smoothly and when a peak was reached a new series of penetrations was made (E_M -79.8 ± 0.5 mV; 11 fibres).

In vivo *measurements*

Thirteen 300 to 400 g rats were anaesthetized with pentobarbitone (50 mg/kg injected intraperitoneally for induction followed by regular intravenous or intraperitoneal injections to maintain an adequate level of anaesthesia). The animals respired spontaneously: on occasion a tracheal catheter was inserted to allow adequate control of the airway. Venous blood samples were taken via a polyethylene catheter in the left superior vena cava and fluid and drugs were infused via a catheter in the left femoral vein.

The animal was placed prone on a Palmer animal heating blanket which maintained the animal's rectal temperature at 37°C (as measured by a rectal thermistor probe). The proximal end of the right tibia and the distal end of the Achilles tendon and tarsus were held rigidly by clamps. The dorsal surface of the soleus muscle was exposed at the bottom of a paraffin oil 'pool' created by tying the edges of the skin incision to a rigid metal ring. Thin tissue paper, moistened with Ringer solution and coated with 1% agar in Ringer was used to extend the skin to deepen the pool. The dorsal muscle surface was bathed by a thin layer of Krebs-Ringer bicarbonate buffer (equilibrated with 5% CO_2 and 95% O_2) which was covered by the paraffin oil to allow gaseous equilibrium between the buffer and the animal's interstitial fluid. The fluid in the pool was maintained at a temperature of 37°C by the passage through it of a polyethylene tube perfused with warm water from a thermostated water bath.

The right sciatic nerve was sectioned proximally in the thigh. A Ag/AgCl reference electrode was buried in the muscle of the left thigh. The E_M of the muscle fibres of the soleus muscle were measured in exactly the same manner as in the *in vitro* experiments.

In five experiments the microelectrode was 'tracked' deeper into the muscle to penetrate muscle fibres down to the fourth layer of fibres. From these experiments it was seen that the mean E_M of the surface fibres was only significantly lower than that deeper in the muscle if the surface fibres were obviously damaged during dissection.

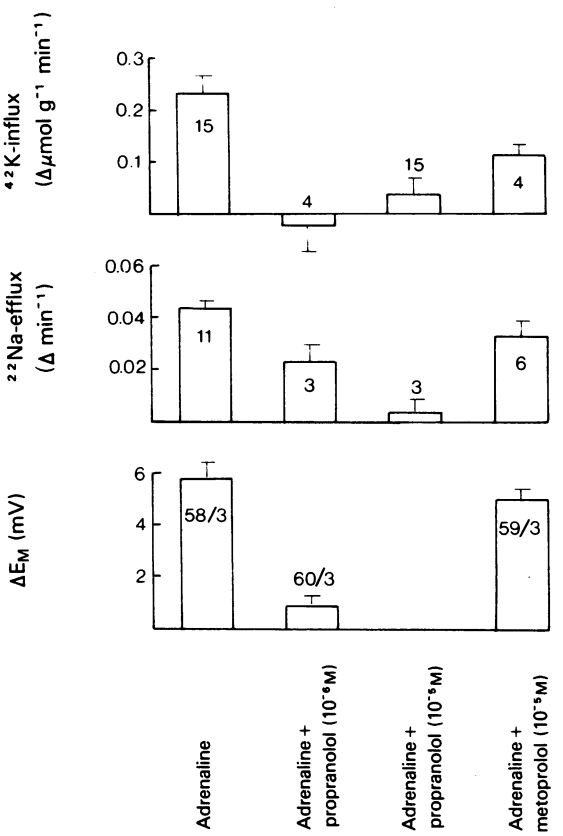

Figure 2 The effects of adrenaline on E_M, ^{22}Na-efflux and ^{42}K-influx and their modification by metoprolol and propranolol. E_M was recorded as described in the legend to Figure 1 and the rise induced by adrenaline (10^{-6} M) determined. The increase in the fractional loss of ^{22}Na was determined within the first 10 min of exposure to adrenaline. ^{42}K-influx was measured using an incubation period of 20 min (see Clausen & Kohn, 1977). When added, propranolol and metoprolol were present from 40 min before the addition of adrenaline. The results are given as means of the difference between control and adrenaline-treated muscles.

All the results of transport and E_M measurements are given as mean values ± standard error of the mean (s.e.). In the figures, s.e. is indicated by bars.

Results

In the rat isolated soleus muscle, adrenaline induces a hyperpolarization, which is associated with stimulation of ^{22}Na-efflux (measured as the fractional loss of isotopic Na from preloaded muscles) and of the ouabain-suppressible component of ^{42}K-uptake

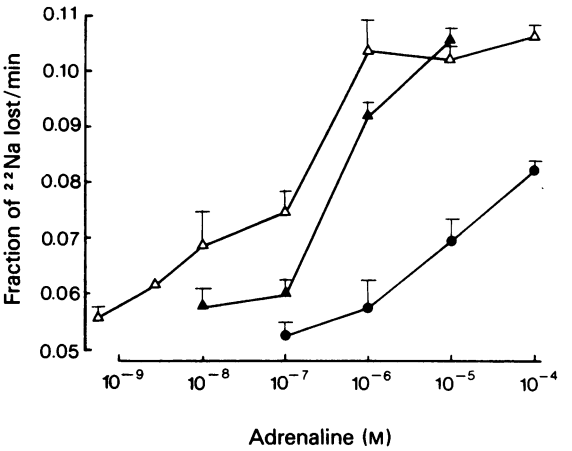

Figure 3 The effect of adrenaline alone (△) on ^{22}Na-efflux and its modification by metoprolol (10^{-5} M, ▲) and propranolol (10^{-5} M, ●). Each point represents the mean of from 3 to 12 observations.

(Clausen & Flatman, 1977). These effects and their modification by β-adrenoceptor antagonists are illustrated in Figure 2. At the concentrations tested, neither propranolol nor metoprolol produced any detectable change in the three parameters when measured in the absence of adrenaline. Propranolol (10^{-5} M) completely abolished the adrenaline-induced increases, where as the β_1-selective antagonist, metoprolol, when added at the same concentration, gave only a partial suppression.

A more detailed comparison of the two antagonists was obtained by testing their effects over a range of adrenaline concentrations (Figure 3). At a dose where the hormone induced half-maximum stimulation of ^{22}Na-efflux, propranolol appeared to be at least 50 times more potent than metoprolol in suppressing the response.

As the hyperpolarizing effect of adrenaline is reversible, it was possible to use the same muscle for a direct comparison of the two antagonists (Figure 4). In a pair of muscles obtained from the same animal, the hyperpolarization evoked by adrenaline (10^{-7} M, muscle B) was considerably suppressed by the presence of propranolol (5×10^{-8} M, muscle A, Figure 4). During a subsequent wash with incubation medium without additions, E_M returned to the level measured before adrenaline. The readministration of the hormone at the same concentration (10^{-7} M) produced virtually the same hyperpolarization (muscle B). In the contralateral muscle (A), pretreatment with metoprolol (10^{-6} M) gave only a marginal reduction in the response to adrenaline. Hence, propranolol seems to be at least 20 times more potent than metoprolol in inhibiting the hyperpolarizing effect of adrenaline.

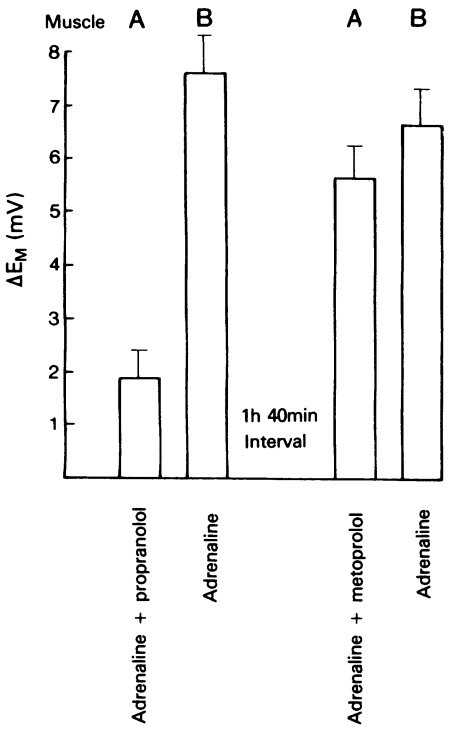

Figure 4 The mean hyperpolarization of a group of muscle fibres exposed to adrenaline, 10^{-7} M, in the presence (muscle A) and absence (muscle B) of 5×10^{-8} M propranolol. The mean changes in E_M (Δ mV) were calculated as in the experiment illustrated in Figure 1. Both muscles were then superfused with drug-free buffer for 1 h and 40 min. The E_M response to adrenaline (10^{-7} M) was then reassessed in the presence (muscle A) and in the absence (muscle B) of 10^{-6} M metoprolol.

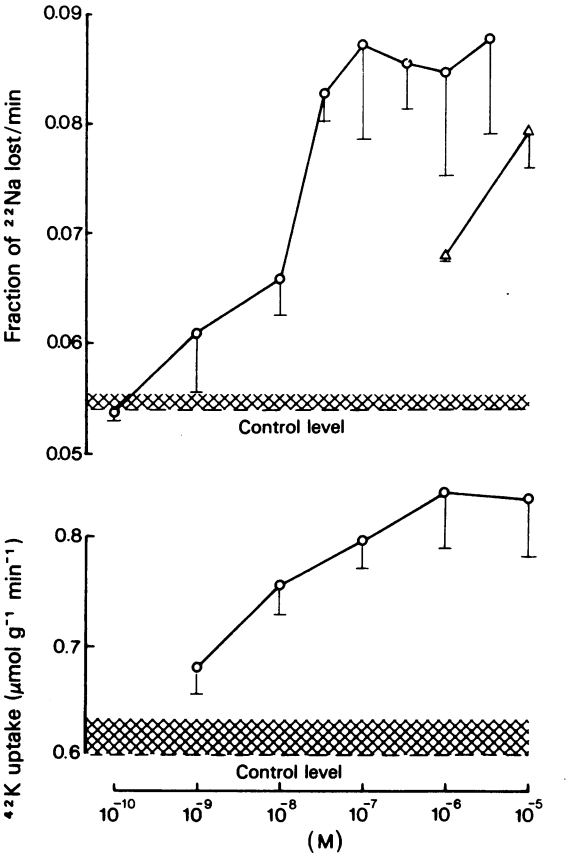

Figure 5 The effect of salbutamol (○) and a β_1-adrenoceptor agonist H133/22, (△) on ^{22}Na-efflux and ^{42}K-influx. The increase in the fractional loss of ^{22}Na was determined within the first 10 min after the addition of salbutamol. ^{42}K-influx was measured during an incubation period of 20 min (see Clausen & Kohn, 1977). Each point represents the mean of from 3 to 8 observations. The cross-hatched area above the control level represents one s.e. mean.

Although propranolol and metoprolol are almost equipotent in suppressing β_1-adrenoceptor-mediated actions in the heart evoked by sympathetic nerve stimulation (Åblad, Carlsson, Ek, 1973), the possibility cannot be excluded that soleus muscles contain β_1-adrenoceptors with a markedly different sensitivity towards the two antagonists. Therefore, as an alternative means of comparing the relative role of β_1- and β_2-adrenoceptors, the effects of β-adrenoceptor agonists were examined. From Figure 5 it can be seen that the β_2-adrenoceptor agonist, salbutamol, on a molar basis is equipotent with adrenaline in stimulating ^{22}Na-efflux (compare with Figure 3). In contrast, the β_1-adrenoceptor agonist H133/22 (−)-1-isopropylamino-3-(*p*-hydroxyphenoxy)-2-propanol-HCl) is at least 100 times less potent in stimulating ^{22}Na-efflux. Whereas the addition of salbutamol at a concentration of 10^{-5} M produced a hyperpolarization of 8.7 ± 0.3 mV (10 muscles, 301 fibres) (see Figure 1), the same concentration of H133/22 only increased the membrane potential by 1.6 ± 0.3 mV (6 muscles, 266 fibres). At 10^{-4} M H133/22 even induced a small depolarization, 1.4 ± 0.6 mV (3 muscles, 102 fibres) indicating that in soleus muscles, β_1-adrenoceptors play a very minor role in mediating the stimulation of active electrogenic Na-K-transport.

In vivo *experiments*

In order to assess the significance of these effects for the control of the resting membrane potential in the intact organism, the action of adrenaline and salbutamol was characterized in a series of experiments on

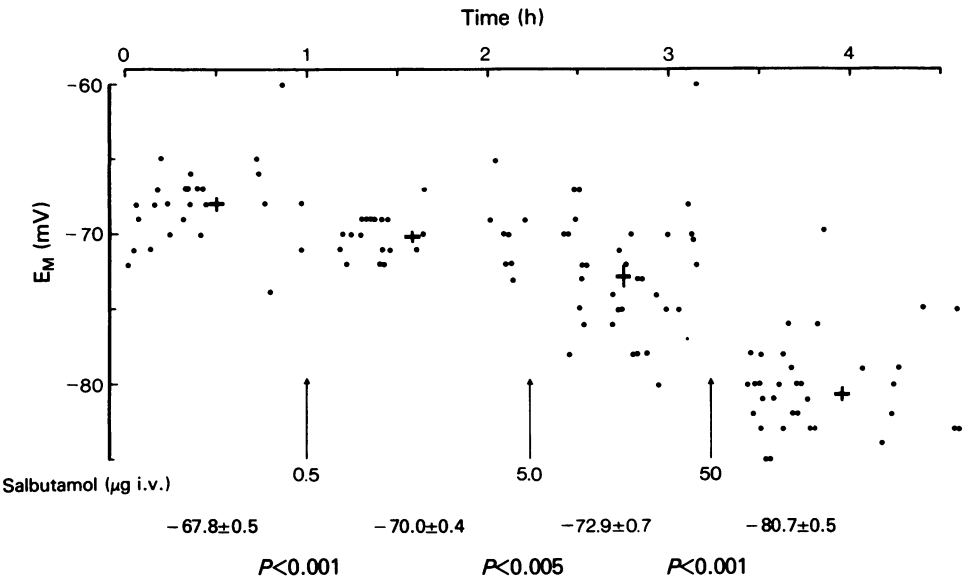

Figure 6 The time course of the effect of salbutamol on the E_M of a soleus muscle (*in vivo*) of a 250 g rat. Each point represents the E_M of a single fibre penetrated at the time indicated. The crosses show the mean E_M (± the s.e. mean) for all the fibres measured during each period delineated by the arrows. Salbutamol (in incremental doses) was injected intravenously over a period of a few minutes at the times shown by the arrows. There was a highly significant difference between the mean E_M of each consecutive group of measurements.

animals anaesthetized with pentobarbitone. Figure 6 shows the effects of intravenous injections of salbutamol on the membrane potential of the exposed soleus muscle. During the control period, E_M was −67.8 mV, slightly lower than the mean value of −71.2 mV obtained in a large series of *in vitro* experiments performed with muscles obtained from rats of the same size (Clausen & Flatman, 1977). The intravenous administration of incremental doses of salbutamol (0.5 to 50 μg; Figure 6) induced a progressive hyperpolarization, which at the highest dose even exceeded the rise obtained with a supramaximal concentration (10^{-5} M) *in vitro* (12.9 mV as compared to 8.7 mV).

In five experiments it was seen that an intravenous injection of 50 μg salbutamol elicited a mean hyperpolarization of 15.3 mV (control E_M: −65.7 + 0.5 mV, 96 fibres; E_M in presence of salbutamol: −81.0 ± 0.7 mV, 111 fibres). It was noted that the rats developed a hyperpnoea following salbutamol infusion. Similar experiments were performed with adrenaline, but this resulted in very active fibrillations in the nearby gastrocnemius muscle which made penetration of the soleus muscle by the microelectrode very difficult. However, in 3 experiments a rise in membrane potential of 11.2 ± 1.0 mV (128 fibres) was seen following an adrenaline dose of 5 μg (i.v.). Moreover, the hyperpolarization was seen in all the 4 muscle fibre layers tested.

Since it is known that salbutamol induces hypokalaemia (Leitch *et al.*, 1976), it was necessary to assess the possible role of decreased extracellular [K] in establishing the hyperpolarization. In an experiment (not illustrated) the intravenous administration of 50 μg salbutamol to a 250 g rat evoked a 20.1 ± 1.5 mV hyperpolarization (49 fibres) whereas the serum [K] fell from 4.5 mM during the control period to 3.9 mM during the peak E_M recorded. In a series of *in vitro* experiments, the potassium concentration of the bathing medium was varied over a range comparable to that recorded in the blood plasma of salbutamol-treated animals. From these experiments (Mølgård, Stürup-Johansen & Flatman, 1980) it was seen that although lowering extracellular [K] causes an increase of E_M, the effect of reducing [K] from 4.5 mM to 3.9 mM (a rise of 1 mV) is only a small fraction of the hyperpolarization observed in the *in vivo* experiments with salbutamol

Discussion

We have shown that the stimulation of active Na-K transport, increase in intracellular K:Na ratio and hyperpolarization produced by catecholamines, can be blocked by the mixed $\beta_1\beta_2$-antagonist, propranolol but not by the α-antagonist, thymoxamine (Clausen

& Flatman, 1977). Hence these actions are probably due to a stimulation of β-adrenoceptors. The stimulation of active electrogenic Na-K transport is also produced by salbutamol, a 'pure' β_2-agonist, so it is probable that the responses are mediated by β_2-adrenoceptors. The results presented here show that the typical stimulation of electrogenic ion transport by β-adrenoceptor agonists is blocked at low concentration by the mixed $\beta_1\beta_2$-antagonist, propranolol, but not by the specific β_1-antagonist, metoprolol. Thus propranolol appears at least 50 times more active at blocking adrenaline stimulation of active Na extrusion than metoprolol (Figure 3).

Similar results were obtained when the antagonism of adrenaline-induced hyperpolarization by propranolol and metoprolol were compared (Figure 2). Propranolol, 10^{-6} M, reduced the adrenaline-evoked hyperpolarization to 14% of its control value, whereas a ten times greater concentration of metoprolol only reduced the adrenaline-induced hyperpolarization to 86%. Also as seen in Figure 4, in the same muscles, propranolol at a concentration 1/20th that of metoprolol, reduced an adrenaline hyperpolarization to 25% of the control whereas metoprolol only reduced the hyperpolarization to 85%. It has previously been shown that metoprolol is four times less potent than propranolol in blocking the β_1-action of isoprenaline on heart rate, and 50 times less potent than propranolol in blocking isoprenaline's β_2-action on vascular resistance (Åblad *et al.*, 1973).

Further evidence that the stimulation of active electrogenic Na-K-transport by catecholamines is mediated by β_2-adrenoceptors was gained from the experiments with the specific β_1- and β_2-adrenoceptor agonists, H133/22 and salbutamol, respectively. Whereas salbutamol is a potent stimulator of active transport and produces large hyperpolarizations at doses similar to adrenaline, H133/22 has a much weaker action on Na-transport (Figure 5), produces only a small hyperpolarization at 10^{-5} M and a 1.4 mV depolarization at 10^{-4} M. Hence it appears that, as in other peripheral tissues the response to adrenaline is mediated via β_2-receptors (see Bowman, 1979). Indeed, whereas H80/62 (the racemate of which H133/22 is the (−) isomer) is over 10^4 times less potent than adrenaline in depressing the sub-tetanic contractions of guinea-pig soleus muscles *in vitro* (Waldeck, 1977), salbutamol has 40% the potency of adrenaline on the same preparation (Olsson, Swanberg, Svedinger & Waldeck, 1979). However, H133/22 has 90% the intrinsic chronotropic β_1-action of isoprenaline on vagotomized and catecholamine-depleted cats (Hedberg, Carlsson & Tångstrand, 1977). Salbutamol has a far greater specificity for β_2 than for β_1-adrenoceptors. The dose-ratio of salbutamol to isoprenaline producing a 50% maximal increase in the rate of the isolated guinea-pig heart (a β_1 action) was 2500:1. On the other hand a dose-ratio of 6:1 was found for the 50% inhibition of the response of isolated guinea-pig trachea to electrical stimulation (Farmer, Kennedy, Levy & Marshall, 1970).

The hyperpolarization evoked by β_2-adrenoceptor agonists *in vitro* (Figure 1) is also seen *in vivo* (Figure 6 and Kendig & Bunker, 1972). Thus the hyperpolarization is not an artifact of isolation of the soleus muscle tissue and the removal of the muscle from its normal hormonal environment. Of course, the decrease in plasma [K] caused by salbutamol would also evoke a hyperpolarization by altering the transmembrane ionic gradients, but this hyperpolarization is considerably smaller than that measured in the experiment shown in Figure 6. Thus in the pentobarbitone-anaesthetized animal, salbutamol produces a hyperpolarization by similar means to the *in vitro* situation and the hyperpolarization is supplemented by the increased transmembrane [K] gradient that can develop *in vivo*.

A similar hyperpolarization of the soleus muscle can possibly also occur when the plasma and interstitial catecholamine concentration is raised due to secretion from endogenous sources. The stimulation of the electrogenic Na-K-transport can be of importance in restoring the normal transmembrane Na and K gradients in muscle tissue during exercise when the extracellular K tends to rise sharply. It is difficult to say whether the hyperpolarization recorded in the soleus muscles on treatment with catecholamines has an important physiological role or whether it is the simple outcome of a stimulation of active Na-K-transport (see Bowman, 1979).

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