J. Pharm. Pharmacol. 1981, 33: 177–179
Communicated October 22, 1980

0022–3573/81/030177–03 $02.50/0

The effect of salbutamol on the membrane potential of normal and depolarized rat diaphragm muscle

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It is known that β-adrenoceptor agonists increase both directly and indirectly elicited responses of fast-contracting skeletal muscles (see Bowman & Nott 1969 for review) and that this effect in the guinea-pig isolated extensor digitorum longus muscle is mediated by β_2-adrenoceptors (Holmberg & Waldeck 1977). Rat isolated diaphragm preparations depressed by the addition of excess potassium chloride to the bathing fluid were greatly sensitized to this twitch potentiating action of sympathomimetic amines and the effects of these amines were antagonized by β-receptor blocking agents (Bowman & Raper 1964).

Hyperpolarization of muscle fibre membranes may contribute to the effect of sympathomimetic amines on muscle twitches but whether these drugs alter membrane potential in this way remains controversial. The adrenaline induced increase in demarcation potential of cat skeletal muscle (Brown et al 1950; Bowman & Raper 1964) would support membrane hyperpolarization. However, results from intracellular microelectrode recordings of skeletal muscle membrane potentials are conflicting. Krnjević & Miledi (1958) found that adrenaline did not affect resting membrane potential in either rat diaphragm muscle in vitro or gracilis muscle 'in situ' and this has been confirmed by Lewis et al (1977) in flexor digitorum longus and soleus muscles of the cat in situ. On the contrary, Kuba (1970) reported a 3–4 mV hyperpolarization of rat isolated diaphragm muscle by adrenaline but the work of Bray et al (1976) has indicated that this muscle is insensitive to the hyperpolarizing effect of adrenaline, noradrenaline and isoprenaline unless it is depolarized as a result of denervation. Salbutamol is a β_2-selective adrenoceptor agonist which is known to increase contractility of rat isolated diaphragm muscle (Farmer et al 1970a) and rat isolated extensor longus digitorum muscle (Al-Jeboory & Marshall 1978), but whether this drug affects muscle membrane potential is not known. The present work was, therefore, done to determine whether salbutamol would hyperpolarize rat diaphragm muscle in vitro and if prior muscle depolarization by potassium chloride would sensitize the muscle to this action. To provide evidence for the type of β-receptor responsible for salbutamol-induced changes in muscle membrane potential the actions of the β-blockers propranolol (non-selective) and atenolol (β_1-selective) have been studied alone and in the presence of salbutamol on membrane potentials of both normal and potassium depolarized fibres.

Experiments were made on rat phrenic nerve diaphragm preparations (Bülbring 1946), removed from white male rats ≈200 g, which had been killed by a blow on the head followed by transection of the cervical spinal cord and set up under the following conditions, which are similar to those described by Liley (1956). Strips of left hemidiaphragm with the phrenic nerve attached were mounted in Krebs-Henseleit solution at 20–22 °C over a Perspex dome in a bath of about 20 ml capacity. The preparations were gassed with 5% CO_2 in O_2 and the bath fluid was continuously exchanged at a constant rate of approximately 250 ml h^{-1}. The composition of the Krebs-Henseleit solution was (mM): Na^+ 143·5, K^+ 5·4, Ca^{2+} 2·5, Mg^{2+} 1·2, Cl^- 128, $H_2PO_4^-$ 1·2, HCO_3^- 24·9, SO_4^{2-} 1·2, glucose 10. Intracellular recordings of muscle fibre membrane potentials were made employing the usual techniques with glass capillary microelectrodes, filled with 3M KCl solution and of resistances in the range 10 to 30 Mohms. After suitable amplification, the membrane potentials were displayed and measured using one beam of a Tektronix Type 502A dual beam oscilloscope. The effect of salbutamol on membrane potentials was observed by first recording resting potentials of fibres under control conditions. The flow of Krebs solution from the reservoir through the bath was then stopped, flow from another reservoir of Krebs solution containing salbutamol started and after allowing time for equilibration (approximately 30 min) membrane potentials were recorded in the presence of the drug. To observe the effect of salbutamol on partially depolarized muscle fibres, Krebs solution to which 0·5 g litre^{-1} potassium chloride had been added was allowed to flow through the bath and, after equilibration, membrane potentials were recorded. Salbutamol was then added to the perfusion fluid in the reservoir and its effect on these potentials was recorded. The effects of propranolol and atenolol on membrane potentials of normal and depolarized muscle fibres were recorded in a similar manner and to investigate any modification of salbutamol effect, these β-blockers, in Krebs solution were allowed to flow through the bath before salbutamol. Drugs used were atenolol, (±)-propranolol hydrochloride and salbutamol sulphate, in concentrations of 83 μM calculated as base.

Drug-induced changes in membrane potentials of normal muscle fibres and of fibres partially depolarized

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Table 1. Effects of salbutamol, propranolol and atenolol on membrane potentials (mV with s.d.) of rat diaphragm muscle fibres in A. Krebs-Henseleit solution and B. Krebs-Henseleit solution plus KCl (0·5 g litre^{-1}).

	A.		
Control (normal Krebs)	Salbutamol		
78·4 s.d. 4·8 (250)	*82·7 s.d. 4·8 (250)		
	Propranolol	Propranolol + salbutamol	
76·5 s.d. 6·2 (100)	76·7 s.d. 4·9 (101)	76 s.d. 5·5 (100)	
	Atenolol	Atenolol + salbutamol	
71·2 s.d. 4·3 (100)	71·3 s.d. 4·6 (120)	*79·3 s.d. 4·4 (147)	
	B.		
Control (normal Krebs)	Krebs + KCl	Salbutamol	
80·2 s.d. 3·8 (100)	60·6 s.d. 3·8 (150)	*65·4 s.d. 3·7 (150)	
		Propranolol	Propranolol + salbutamol
73·7 s.d. 4·6 (100)	56 s.d. 4·4 (100)	56·1 s.d. 4·2 (100)	57·1 s.d. 2·8 (100)
		Atenolol	Atenolol + salbutamol
74 s.d. 2·9 (100)	59·3 s.d. 3·8 (100)	59·5 s.d. 3·4 (100)	*73·4 s.d. 3·9 (100)

* = Significant differences ($P < 0·001$) from values in either normal Krebs solution or in Krebs + KCl solution. Number of fibres in brackets. Drug concentrations—83 μM.

by potassium chloride are shown in Table 1, where the potentials marked by asterisks represent statistically significant changes using Student's *t*-test. Potassium chloride depolarized the muscle fibres from a mean value (with s.d.) of 75·9 (3·8) mV (300 fibres) to 58·6 (4) (350 fibres). This depolarization was expected and follows the reduction of the concentration gradient across the cell membrane for potassium ions. Salbutamol hyperpolarized muscle fibres in normal Krebs solution by approximately 4–5 mV and repolarized fibres partially depolarized by potassium chloride by a similar amount. The β-blockers propranolol and atenolol had no effect on the membrane potential of either normal or potassium depolarized fibres. However, propranolol blocked the effect of salbutamol in both cases, whereas atenolol did not, and in fact the effect of salbutamol was greater in the presence of atenolol, especially in muscle fibres partially depolarized by potassium chloride. It, therefore, seems that activation of β_2-receptors by salbutamol is responsible for the increase in muscle fibre membrane potential and it is known that β_2-receptor excitation leads to increased muscle twitch (Holmberg & Waldeck 1977). The increased effect of salbutamol on membrane potential in the presence of atenolol may be due to sensitization of skeletal muscle β_2-receptors by the latter drug to the hyperpolarizing action of the β-agonist and perhaps easier access of atenolol to the receptor sites in potassium-depolarized fibres explains the greater degree of salbutamol-induced hyperpolarization in these fibres.

It may be that drug-induced increase in membrane potential contributes to changes in muscle contractility but as this hyperpolarization was small and of approximately the same degree in normal and depolarized fibres it seems an unlikely explanation for the increased effect of β-receptor agonists on the contractility of potassium depolarized muscle. The mechanisms underlying change in contraction pattern induced by these drugs is incompletely understood but Bowman & Nott (1969, 1974) suggested a cyclic (c)AMP mediated effect on the availability of calcium ions for the contractile process. They also believe it is possible that membrane depolarization by potassium allows easier drug access to the sarcoplasmic reticulum site of adenyl cyclase and this may explain the sensitization of rat diaphragm muscle to β-receptor agonists. The effect of salbutamol on membrane potential of skeletal muscle fibres is probably due to increased cAMP production which the drug is known to induce in skeletal muscle (Al-Jeboory & Marshall 1978) and consequent triggering of events which lead to changes in transmembrane ion fluxes. If the membrane potential of rat diaphragm muscle is adequately explained by passive ionic permeabilities of the fibre membrane, primarily by the ratio $\{K\}_i:\{K\}_o$ as suggested by Wareham (1978) for rat soleus and extensor digitorum longus muscle, then an alteration of membrane permeability to this ion may be the main action of salbutamol. However, if there is an electrogenic component to the membrane potential of rat diaphragm muscle, albeit of major importance when the muscle is denervated (Bray et al 1976) then Na^+ pump stimulation may be a contributing factor.

We thank Mr J. A. Tweed for arranging financial help and a gift of atenolol from Stuart Pharmaceuticals Ltd. Salbutamol sulphate was kindly donated by Glaxo-Allenburys Research Ltd.

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J. Pharm. Pharmacol. 1981, 33: 179–182
Communicated September 8, 1980

0220–3573/81/030179–04 $02.50/0

A model for the pH dependence of drug-protein binding

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Change of pH alters the protein binding of drugs (Goldbaum & Smith 1954; Newbould & Kilpatrick 1960; Burney et al 1978; Vallner et al 1979). Since the free or unbound portion of a drug is generally held to be responsible for its pharmacological effects, we have sought ways of expressing pH-induced change in free concentration in practical terms. Because the more highly protein bound drugs tend to be lipophilic (Bird & Marshall 1967; Helmer et al 1968; Hansch & Dunn 1972; Chien et al 1975), we began by considering pH induced changes in relation to pH-partition theory.

In its simplest form, this theory applies to the distribution at equilibrium of a weak acid or base between two immiscible solvents, in one of which, the aqueous phase, it is partly ionized, and in the other, the lipid phase, it is non-ionized. Non-ionized solute distributes between the phases in a concentration ratio which defines the true partition coefficient (TPC) of the substance. The extent of the ionization in the aqueous phase is determined by the pK_a of the substance and pH, and these, together with the TPC, therefore govern the distribution of the solute between the phases. Fig. 1 is a computer generated plot of the concentration in the aqueous phase for substances obeying the theory, and shows the amount in the aqueous phase as a percentage of the total amount in the system, and how this varies with pH and with the TPC for a given substance. It illustrates how it is theoretically possible for large changes in concentration to occur over a narrow pH range for substances with the appropriate physiochemical characteristics. We attempted to apply this model to the partitioning of drugs between aqueous buffers and organic solvents, but a model which also allowed for the partitioning of ionized drug into the lipid phase gave an improved fit. The derivation of both of these models is given in Appendix 1.

Extension of this theory to a system such as drug-protein binding requires taking account of the fact that protein molecules are dissolved in the plasma, and therefore bound drug must be regarded as being in the lipid phase and unbound drug in the aqueous phase. In the case where non-ionized solute only binds to a single site on a single species of protein molecule, the curves in Fig. 1 are equally applicable (see Appendix 2). In this case, a considerable concentration change of free drug with pH would only occur if the drug had a high protein affinity and was in the presence of an adequate amount of protein. In vivo partitioning from tissues

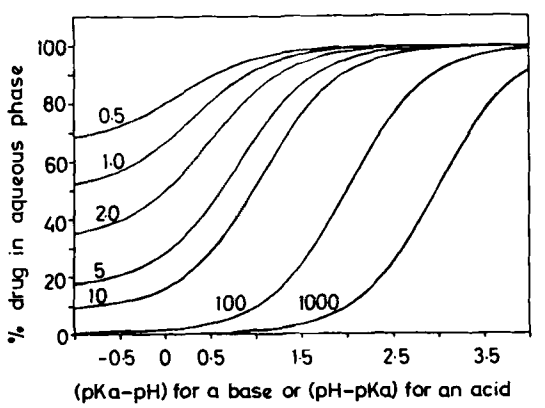

FIG. 1. Plots of the predicted concentration in the aqueous phase (expressed as a percentage of the maximum) of solutes which obey the simple pH-partition theory shown as a function of pH. Each curve is drawn using the specified value for K_u, which is the quantity ratio or volume corrected true partition coefficient for a given solute (see Appendix 1, eqn 6).

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