Bimodal effect of glycine on frog spinal motoneurones

R.H. EVANS* & J.C. WATKINS

Department of Pharmacology, University of Bristol, Medical School, Bristol BS8 1TD

Glycine has been shown to have variable effects on the polarity of amphibian motoneurones as recorded from ventral roots of the isolated hemisected spinal cord (Tebecis & Phillis, 1969; Barker & Nicoll, 1973). In the present experiments regenerative activity in isolated frog hemicords was blocked with procaine which allowed the direct effect of glycine on the polarity of motoneurones to be measured. In such preparations it was found that glycine evoked either hyperpolarization or depolarization depending on the composition of the Ringer solution bathing the cord.

The standard Ringer solution contained (mM):– NaCl, 111; KCl, 2; $CaCl_2$, 2; Tris base, 10; glucose, 12; procaine HCl, 1; adjusted to pH 7.9 with HCl. With this solution, glycine (0.25-2 mM) regularly produced depolarization. Chloride-deficient Ringer ($NaMeSO_4$, 111 mM; $KMeSO_4$, 2 mM; $CaSO_4$, 2 mM) enhanced the depolarization caused by glycine. However, in potassium deficient (KCl, 0.5 mM) and sodium deficient (NaCl, 22 mM; choline chloride, 88 mM) Ringer solutions the depolarization caused by glycine was diminished and hyperpolarization often occurred.

Glycine also produced hyperpolarization when tested in the presence of a depolarizing concentration of L-glutamate (0.2-0.5 mM). The reversal of the response to glycine produced by L-glutamate could not have been due to depolarization alone because similar depolarizations produced by potassium chloride (4-10 mM, total KCl) did not cause glycine to hyperpolarize.

Strychnine (50 μM) increased glycine-induced depolarizations and converted glycine-induced hyperpolarizations into depolarizations.

Thus it appears that glycine has a strychnine-sensitive hyperpolarizing action and also a depolarizing action which may be mediated by different receptors on the motoneurone. It is probable that the hyperpolarizing receptor is activated by β-alanine and taurine in addition to glycine because these amino acids also have hyperpolarizing actions which are blocked by strychnine.

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Extracellular and intracellular effects of procion brilliant red H3BN on mammalian muscle fibres

J.B. HARRIS & R.R. RIBCHESTER*

Muscular Dystrophy Group Research Laboratories, Regional Neurological Centre, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, England

The interpretation of the physiological and pharmacological properties of muscles from animals suffering from the degenerative diseases of muscle is hindered by the occurrence of severely damaged and normal fibres within the same muscle. One approach to overcoming this obstacle would be to mark individual fibres with a stable dye during an experiment and subsequently to isolate these fibres for morphological and histochemical examination.

The Procion dyes (Stead, 1973), and particularly Procion Yellow, have been widely used for marking nerve cell processes, but not extrafusal muscle fibres. Procion dyes are known to bind to cellular protein (Flanagan, Hesketh & Chung, 1974) but the possibility that they may alter the physiological characteristics of the exposed or injected cells has not been examined. It has, however, been suggested that Procion Yellow has local anaesthetic properties (Payton, 1970).

We have examined the effects of extracellular and intracellular Procion dyes on some properties of mammalian muscle. Procion Yellow MX4R, Procion Navy Blue H3R and Procion Brilliant Red H3BN have all been tested, but only Procion Brilliant Red was found to be routinely suitable for intracellular injection.

Experiments were carried out on isolated extensor digitorum longus, soleus and diaphragm muscles of normal rats and mice. The muscles were maintained *in vitro* at room temperature. The

presence of Procion Brilliant Red (1% w/v; -5×10^{-3} M) in the bath had no effect on membrane potential, miniature end-plate potentials, 'input resistance', or action potentials elicited either by indirect stimulation or direct excitation of individual muscle fibres, 1 h after its application.

In another series of experiments individual muscle fibres were injected with Procion Brilliant Red by passing hyperpolarizing direct currents of 40 nA through an intracellular micropipette containing a 4% (w/v) solution of the dye. In these experiments the dye-filled micropipette was used as the current passing electrode when measuring 'input resistance' or generating muscle fibre action potentials. Action potentials were measured immediately following penetration of the dye-filled micropipette and again 1-2 min later.

In many fibres dye injection was associated with a localized swelling of the muscle fibre and sometimes this was accompanied by a rapid fall in membrane potential. In most fibres, however, there was no significant change in any measured parameter. Satisfactory marking of the muscle fibres was attained within 15-20 s of penetration.

The results suggest that Procion Brilliant Red H3BN is a suitable agent for marking individual muscle fibres, and within the context of these experiments, is devoid of appreciable pharmacological activity.

Details of the techniques used to prepare the micropipettes, and of the techniques of injection and post-injection fixation are described elsewhere (Harris & Ribchester, 1975).

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The mechanism of 'adrenaline reversal' in the anaesthetized cat and rabbit

H.C. GRUNDY* & H.F. GRUNDY

Westminster Medical School, London and Department of Pharmacology, University of Cambridge

α-Adrenoceptor blockers share the ability to produce adrenaline reversal in anaesthetized laboratory animals (Dale, 1906; Nickerson, 1949). Adrenaline activates vasoconstrictor (α-) and positive inotropic and chronotropic (β_1-) adrenoceptors which mediate pressor responses, and vasodilator (β_2-)adrenoceptors which are depressor. Therefore, α-blockade will only produce adrenaline reversal if the β_2-effect transcends the β_1-action. This could result from three possibilities:– (1) the β_2-action intrinsically exceeds the β_1-effect; (2) the β_2-response is potentiated by α-blockade; (3) the α-blocker antagonizes the β_1-action.

We examined these hypotheses in 41 adult cats anaesthetized with i.p. chloralose (80 mg/kg) or sodium pentobarbitone (35 mg/kg). In 17 cats, by selective blockade with phentolamine, practolol or propranolol, we determined the contributions of α-, β_1-, and β_2-adrenoceptor responses to the BP changes elicited by i.v. noradrenaline, adrenaline or isoprenaline doses up to 10 μg/kg. The log dose-response curves showed for adrenaline:– firstly, the β_1-threshold is highest; secondly, the β_2-effect often predominates at low doses giving depressor actions but at higher doses the α- and β_1-effects cause a pressor response; thirdly, at all doses and in all cats the β_2-action exceeds the β_1-effect. The α- and β_1-components of noradrenaline are similar to those of adrenaline but β_2-activation is weak (see later). Isoprenaline shows β-effects greater than those of adrenaline: but the α-blocker left the isoprenaline response unchanged which indicates that phentolamine has little significant action upon β-components. In further experiments, using ten different α-blockers, we found that neither β_1-depression nor β_2-potentiation provides a satisfactory explanation for adrenaline reversal.

Adrenaline reversal is invariably associated with a marked degree of vasodilator (β_2-) stimulation.