Receptor-specific binding of [$^3$H]-atropine was present in both the caudate nucleus and cerebral cortex of 5-day-old rats in concentrations which were 20–30% of the adult values. The concentrations increased with age and by 16 days had reached approximately 60% of the adult concentrations of receptor sites which were attained by the fifth week of life. Throughout, the caudate nucleus contained a higher concentration of receptor sites than the cerebral cortex.

These results indicate that the lack of response to scopolamine in young rats is not a consequence of the absence of the muscarinic cholinergic receptor though it is possible that the receptor is not functionally connected to the processes which are responsible for mediating the changes in neuronal activity produced by cholinergic agents.

I am indebted to Caroline Middleton for skilful technical assistance.

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## Analysis of end-plate current fluctuations produced by acetylcholine and acetylmonoethylcholine in rat muscle

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The characteristics of the ion channels that are opened by agonists in the frog muscle end-plate have been studied by analysis of the fluctuations about the mean end-plate voltage, or current, produced by the drugs (Katz & Miledi, 1972; Anderson & Stevens, 1973; Colquhoun, Dionne, Steinbach & Stevens, 1975). Similar experiments have now been conducted on rat diaphragm muscle fibres (Sakmann, 1976).

Strips of rat diaphragm were bathed in Krebs' solution containing tetrodotoxin (300 nM), at temperatures between 13° and 25°C. End-plate currents were recorded by a voltage clamp method. Analysis of current fluctuations was carried out on 10 s samples, filtered below 0.5 Hz and above 500 Hz. Digital samples were taken at 1 ms intervals. Agonists were applied by perfusion from a wide tipped micropipette (Cooke & Quastel, 1973) so as to produce end-plate currents of 10–90 nA.

The decay of spontaneous miniature end-plate currents (MEPC) showed no consistent deviation from a single exponential; and, equivalently, spectral density curves for both acetylcholine (ACh), and for acetylmonoethylcholine (AMECh) showed only slight deviation from a single Lorentzian form, so a single time constant could be inferred. The time constants for both MEPC decay ($\tau_{MEPC}$) and for current fluctuations ($\tau_{ACh}$ or $\tau_{AMECh}$) decreased with temperature ($Q_{10} = 3.26 \pm 0.63$), and increased as the membrane was hyperpolarized ($109 \pm 7$ mV for an e-fold change in $\tau$). These values are similar to those for frog muscle.

At 20°C, and −80 mV membrane potential, $\tau_{MEPC}$ was 1.6 ms. This appeared to be slightly (20–50%) slower than $\tau_{ACh}$, though the size of the discrepancy depended on the method of curve fitting used for spectra. The simplest, but not the only, explanation for such a discrepancy would be that the acetylcholine concentration in the synaptic cleft did not fall rapidly compared with the lifetime of an open channel.

The ratio $\tau_{AMECh}/\tau_{ACh}$ was $0.56 \pm 0.05$ (15); this can be interpreted as meaning that the average lifetime of an open ion channel, when the channel is opened by AMECh, is about 56% of its mean lifetime when opened by ACh. This ratio is similar to the ratio of decay time constants for normal (ACh) and 'false' (AMECh) MEPCs observed by Large & Rang (1976).

The mean conductance ($\gamma$) of a single open ion channel, may, under certain assumptions, be inferred from the variance of current fluctuations. No evidence was found for a difference in conductance between channels opened by ACh and AMECh; the values were $\gamma_{ACh} = 24.9 \pm 1.2$ pS ($n = 36$) (very similar to the value reported for frog muscle by Colquhoun *et al.*, 1975), and $\gamma_{AMECh} = 26.7 \pm 1.4$ pS ($n = 16$).

I am indebted to Dr Colin Mercer, Institute of Sound and Vibration Research, Southampton University, for data analysis facilities.

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## Pharmacological agents and acute experimental hyperlactataemia in the dog

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Experimental hyperlactataemia was produced in dogs anaesthetized with pentobarbital 30 mg/kg i.v.: (a) by administration of high doses of biguanides (phenformin 30 mg/kg intraduodenally, metformin 150 mg/kg intraduodenally) (Loubatières, Ribes & Blayac, 1973); (b) by bilateral repeated and prolonged electrical stimulation of the sciatic nerves (unidirectional and rectangular pulses of 10V, 5 ms and 60 Hz) producing repeated and prolonged contractions (60 min) of the gastrocnemius and soleus groups of muscles; (c) by hypoxia following closed circuit inhalation of a mixture of oxygen (9%) and nitrogen (91%) and (d) by continuous i.v. injection of adrenaline (1.5 $\mu g\ kg^{-1}\ min^{-1}$).

An autoanalyser was used to make continuous measurement of blood sugar in haemolysed blood with potassium ferricyanide and plasma lactate with an enzymatic method (Hohorst, 1963; Minaire, Studievic & Foucherand, 1965). Blood lactate and pyruvate were estimated in fractioned samples by the enzymatic method described by Czok & Lamprecht (1970) and blood pH was measured with a KCl electrode.

Insulin, when administered i.v. (0.085 u $kg^{-1}\ h^{-1}$) or produced *endogenously* by i.v. injection of the hypoglycaemic sulfonamide, glibenclamide (0.05 mg/kg) (Loubatières & Mariani, 1967) prevented, delayed or reduced the hyperlactataemia, hyperpyruvicaemia and acidosis which normally followed the administration of biguanides (Loubatières, Ribes & Blayac, 1973). Co-carboxylase (5 mg $kg^{-1}\ h^{-1}$) produced the same effect (Valette, Ribes, Rondot, Loubatières-Mariani & Loubatières, 1975).

The administration of sodium dichloroacetate (30 mg $kg^{-1}\ h^{-1}$) produced a reduction in the hyperlactataemia produced by phenformin (from $50.6 \pm 10.2$ mg/100 ml to $14.3 \pm 2.8$ mg/100 ml, $n = 13$), by intense muscular work (from $42.3 \pm 10.3$ mg/100 ml to $9.0 \pm 1.5$ mg/100 ml, $n = 3$), or by adrenaline (from $61.3 \pm 13$ mg/100 ml to $29 \pm 2.3$ mg/100 ml, $n = 3$). On the other hand, dichloroacetate did not reduce the hyperlactataemia produced by hypoxia (from $84.7 \pm 12.1$ mg/100 ml to $100.8 \pm 8.7$ ml, $n = 3$). These measurements of lactate were made in total blood.

Dichloroacetate slightly reduced the increase in lactataemia which can be observed in the dog presenting a permanent diabetes following the injection of alloxan (50 mg/kg i.v.). Glycaemia was not notably modified.

The therapeutic implications of these observations are under study.

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