output caused by theophylline (Goldberg & Singer, 1969) as such, since adenosine appears to reduce transmitter output by approximately the same extent whatever its original value.

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Accumulation of calcium at the motor endplate

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Denervated rat diaphragm muscle shows an increase in uptake of ^{45}Ca in the presence of acetylcholine (ACh) which does not depend on membrane potential (Jenkinson & Nicholls, 1961). In addition, histochemical studies with the dye Alizarin red S seem to indicate that calcium accumulates at the motor endplate regions of mouse diaphragms treated with ACh (Lièvremont, Czajka & Tazieff-Depierre, 1968). This paper describes experiments which suggest that ACh and carbachol specifically influence the entry of calcium to the motor endplate region.

The influx of calcium at the motor endplate region of the mouse diaphragm, and its dependence on the presence of ACh or carbachol was examined, by means of ^{45}Ca. Mouse diaphragms which had been incubated at 37°C in Ringer solution (Lièvremont, *et al.*, 1968) which contained ^{45}Ca were dried in acetone and cleared in xylene, and the non-innervated and innervated zones of the muscle were separated. The radioactivity of ^{45}Ca in the muscle samples was determined by liquid scintillation counting, and the amount of calcium accumulated at the motor endplate region was calculated as nmol/diaphragm.

Probably because of hydrolysis, at least 0·5 mM ACh was required to produce a significant effect on the influx of calcium, but carbachol was effective in lower concentrations, and therefore it was used to demonstrate the time course of the accumulation of calcium at the motor endplate region. In the presence of carbachol, calcium accumulation increased progressively for 60 minutes. The initial rate of accumulation of calcium was $6{\cdot}20 \pm 0{\cdot}59$ (S.E. of mean) $\times 10^{-12}$ mol of calcium/motor endplate per hour (4998 ± 204 S.E. of mean, muscle fibres/hemidiaphragm). Calcium also accumulated at the endplates of diaphragms which had been denervated 18 days before incubation with ^{45}Ca Ringer solution and ACh.

To measure the efflux of accumulated calcium from the motor endplate region, diaphragms were pretreated for 30 min with 1 mM ACh in ^{45}Ca Ringer solution. ACh (1 mM) reduced the rate of efflux of the accumulated ^{45}Ca from the motor endplate region into tracer-free Ringer solution. The half time of washout for ^{45}Ca from diaphragms into calcium-free Ringer solution was 65 min for the calcium accumulated at the motor endplate region and 15 min for the calcium which had entered the non-innervated zone of the muscle.

These preliminary experiments show an uptake and binding of calcium by the motor endplate region of the muscle in the presence of carbachol and ACh. During normal motor nerve activity, it is unlikely that calcium accumulates in the quantities which have been demonstrated here, but it does seem possible that some calcium may be bound at the motor endplate during the transmission of each impulse.

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Pharmacological interaction of lorazepam with thiopentone sodium and skeletal neuromuscular blocking drugs

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Lorazepam (Wy 4036; 7-chloro-5-(*O*-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one) is a highly active tranquillizing drug (Haider, 1971; Turner & Harry, 1971) which also has an application in preanaesthetic medication (Norris, 1971). In view of the recent reports by Feldman & Crawley (1970a, b) of a hazardous anaesthetic interaction of diazepam, experiments have been performed to investigate the pharmacological interactions of lorazepam with thiopentone and skeletal neuromuscular blocking drugs.

In mice infused intravenously with thiopentone sodium, oral doses of lorazepam (0·1–10 mg/kg) reduced the time to induction of hypnosis and arrest of respiration. Furthermore, the duration of the loss of righting reflex after a single intravenous injection of thiopentone (50 mg/kg) was increased by lorazepam in doses of 1·25–6·25 mg/kg. Comparative tests with diazepam, meprobamate and glutethimide showed a similar enhancement of the effects of thiopentone. To investigate whether these effects represented a potentiating or an additive action of lorazepam with thiopentone, the effect of various dose combinations of the drugs on loss of righting reflex in mice was examined. The results indicated that lorazepam and thiopentone interacted to produce an effect greater than that expected from simple addition.

Experiments in decerebrate cats in which contractions of the gastrocnemius muscles elicited by stimulation of the sciatic nerves were measured, showed that intravenous injection of lorazepam (0·5 and 2 mg/kg) or diazepam (0·5 and 2 mg/kg) failed to alter significantly the responses either to intravenous suxamethonium (10–50 μg/kg) or to intravenous gallamine (100–500 μg/kg).

These animal experiments have shown no untoward pharmacological interactions when lorazepam or diazepam are combined with thiopentone or skeletal neuromuscular blocking drugs.

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