choline induced contractions; on both these preparations, piperazine unlike (+)-tubocurarine produced a block which was slow in onset and non-competitive. Piperazine also produced a slow, dose dependent contracture of the frog and leech muscles.

These results indicate that the neuromuscular blocking activity of piperazine in the species studied is different from that of (+)-tubocurarine.

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Methods for investigating barbiturate tolerance

I. H. STEVENSON and M. J. TURNBULL, *Department of Pharmacology and Therapeutics, University of Dundee, Dundee, DD1 4HN*

Rats may become tolerant to barbiturate during exposure to barbiturate (tolerance) or non-barbiturate (cross-tolerance) drugs. One simple, and most often used, measure of tolerance is the reduction in response, usually the duration of loss of righting reflex to intraperitoneally administered barbiturate. However, determination of barbiturate sleeping time gives no information on the relative contribution of adaptation by the central nervous system and stimulation of the rate of drug metabolism to the overall tolerance. Our tolerance studies indicate that the contribution of central nervous tolerance is small. Indeed, the tolerance which develops to metabolized barbiturates, such as hexobarbitone or pentobarbitone, appears to be entirely accounted for by stimulation of hepatic drug-metabolizing enzyme activity and in our experience, when sleeping time is determined after intraperitoneal injection of barbitone, which is not metabolized ($<5\%$), it is not possible to demonstrate the presence of tolerance even in barbitone dependent rats.

We have found that useful information on the sensitivity of the brain to barbiturate can be obtained by determination of sleeping time following the injection of pentobarbitone sodium into the lateral cerebral ventricles. In this demonstration results will be presented of experiments designed to test the validity of this method for estimating the sensitivity of the central nervous system to barbiturate. Our findings obtained with rats chronically treated with, and withdrawn from, drugs such as morphine, alcohol, barbitone and nitrazepam will be shown. In some experiments the brain level of barbiturate on awakening after the injection of labelled pentobarbitone by this route was measured.

When, in addition, the following estimations are made (a) sleeping time after intraperitoneal injection of labelled barbiturates; (b) brain, liver and serum levels of labelled drug and metabolites on awakening; (c) the capacity of liver microsomal preparations to metabolize labelled barbiturates *in vitro*, a more complete assessment of the tolerance mechanisms operating is possible.

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