These nucleoside-protein complexes may contribute to the formation of receptor sites in postsynaptic membrane, and they may also be involved in storage sites for acetylcholine and catecholamines and in transport mechanisms in the presynaptic membrane. Several molecular models of such 'receptors' as 'storage sites' will be shown together with specific predictions that have been made from the hypothesis that can be tested by experiment.

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Comparison of the neuromuscular blocking action of (+)-tubocurarine and piperazine in the leech, frog, rat and cat

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Norton & de Beer (1957) investigated the mechanism by which piperazine paralysed *Ascaris lumbricoides* muscle, and found that acetylcholine induced contractions of the parasite; these were antagonized by piperazine. They concluded that piperazine acts as a myoneural blocking agent in this species. They also found that piperazine has virtually no blocking activity on mammalian muscle. (+)-Tubocurarine has a weak neuromuscular blocking activity on *Ascaris* muscle compared with that on mammalian muscle. Frog muscle appears to occupy an intermediate position between *Ascaris* and mammals since its response to acetylcholine is readily blocked by both piperazine and (+)-tubocurarine (Bueding, 1962). We therefore decided to investigate the relative neuromuscular blocking activity of piperazine and (+)-tubocurarine in the cat, rat, frog and leech.

Responses of the following muscles were recorded:

1. The anaesthetized cat anterior-tibialis and gastrocnemius muscles stimulated through the sciatic nerve.
2. The anaesthetized rat hind limb muscles stimulated through the sciatic nerve.
3. Rat isolated phrenic nerve-diaphragm muscle at 37° C in Krebs solution.
4. Frog isolated rectus abdominis muscle at 22° C in frog Ringers.
5. Frog isolated gastrocnemius-sciatic nerve at 22° C in frog Ringers.
6. Leech isolated dorsal body wall muscle at 22° C in leech Ringers (eserinsed 1 μg/ml).

The neuromuscular blocking activity of (+)-tubocurarine was similar in each species tested. However, the myoneural blocking action of piperazine differed; it was weakest in mammals, and strongest in leech.

In the rat phrenic nerve-diaphragm preparation, piperazine causes a direct muscle depression (Aubry, Cowell, Davey & Shevde, 1970), and this was confirmed. In the rat and cat *in vivo* experiments, no depression of the muscle twitch was found, but a potentiation of the muscle twitches was recorded.

In the frog gastrocnemius preparation, the effects of nerve stimulation, but not those of muscle stimulation, were selectively inhibited by piperazine. In the frog rectus abdominis preparation, and leech body wall preparation piperazine reduced acetyl-

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

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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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