DEMONSTRATIONS

Method for the quantitative wash-out of the stomach in the anaesthetized rat

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Quantitative wash-out of the stomach of the rat in the Ghosh & Schild (1958) preparation is often difficult to achieve. This is because rats' stomachs are full of solid material even after food has been withheld for 48 h and because in the anaesthetized rat the stomach does not drain completely. In order to overcome these difficulties rats were placed in a metabolism cage (to prevent cophrophagy) 48 h before the experiment and fed on a low residue diet (Complan, Glaxo and milk on the first day and lumps of sugar on the second day). On this regime the stomachs contained no solid material. During the experiment 3 ml of saline were left in the stomach for 15 min and then removed by injecting air and saline alternately. The completeness of wash-out was investigated by adding phenol red (phenyl sulphonphthalein) to every alternate 3 ml sample left in the stomach and measuring the recovery after 15 min and after the subsequent 15 min when no extra dye had been added. The results are shown in Table 1.

TABLE 1. *Percentage recovery of phenol red from the stomach of anaesthetized rats (a) 15 min after adding phenol red, (b) at the end of the subsequent 15 minutes*

Expt.	No of observations	(a) Mean S.D.	(b) Mean S.D.
1	6	91·3±4·3	5·6±3·5
2	9	88·2±6·1	11·5±2·8
3	6	92·5±2·5	3·7±3·2
4	7	96·0±5·7	4·0±6·8
5	6	90·0±5·5	4·7±3·6
6	5	96·3±3·5	3·7±2·3
Mean		92·3±4·6	5·5±3·7

REFERENCE

GHOSH, M. N. & SCHILD, H. O. (1958). Continuous recording of acid gastric secretion in the rat. *Br. J. Pharmac. Chemother.*, **13**, 54–61.

A possible role for nucleoside-protein complexes in membrane

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The hypothesis has been presented (Smythies, 1971) that complex formation between various phosphorylated nucleosides and protein may play an important role in synaptic function. This is based on the molecular complementarity between guanine (G) and glutamate (glu), cytosine (C) and arginine (arg), adenine (A) and glutamine ($gluNH_2$), and uracil and glutamine, which allows the possibility of forming these Watson-Crick-like ion-dipole or hydrogen bonded aminoacid-base pairs (Fig. 1). Abood & Matsubara (1968) have shown that ATP binds strongly to a protein extracted from rat brain synaptosomes probably by electrostatic bonds to glutamine or asparagine moieties. The G-glu, C-arg and A–$gluNH_2$ pairs are all approximately of the same length and could form ladder-like complexes if the synaptosome protein

concerned had a β conformation and one of these specific amino-acids in every alternate locus.

Molecular models indicate that certain of these complexes in turn provide potential binding sites for prostaglandins (PG) since they contain polar groups in complementary loci and with correct bond angles to form hydrogen bonds with the polar groups on the PGs (as well as lipophilic contacts). For example, PGF could bind: $-COO^-$ and 9 OH to amide NHs, 11 OH to the spare electron pair of glutamate 0 and 15 OH from guanine NH spare proton as indicated in the figure. These complexes require to be stabilized by divalent ions such as calcium.

By varying the amino-acid sequence in the protein (which in turn determines which nucleosides and which PGs would bind) a number of different complexes result. Molecular models indicate that some of these could be the receptor sites for various transmitters, since they satisfy the stereochemical requirements for binding the transmitters, their agonists and antagonists.

The amino-acid sequences for model receptors for all putative transmitters except histamine have been specified to satisfy these criteria. The formation and disruption of these complexes may control the ionic permeability of the membrane by altering its 'packing'. Disruption of the complex (which is postulated to be the function of transmitters) would also liberate PGs, metabolically important nucleosides and calcium ions.

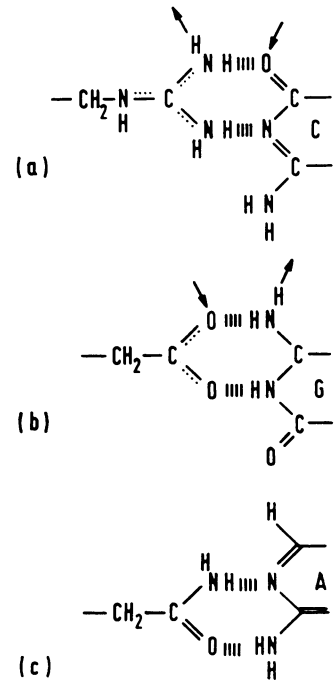

FIG. 1. Complementary aminoacid-nucleoside pairs
(a) Arginine-cytosine
(b) glutamate-guanine
(c) glutamine-adenine
The bond angles on (a) and (b) are shown.

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-