e.g. oxotremorine-M, carbamoylcholine, (+)-acetyl-β-methylcholine and acetylcholine, that produced large elevations of cyclic GMP levels and weaker agonists e.g. arecoline, oxotremorine, pilocarpine that produced no detectable elevation of cyclic GMP levels. The weaker agonists did, however, interact with the receptor since pilocarpine blocked the carbamoylcholine-induced cyclic GMP elevation and all the agonists tested were found to bind to the receptor in ligand binding studies (Strange, Birdsall & Burgen, 1978).

Blume, Chen & Foster (1977) have reported that phosphodiesterase inhibitors can prevent certain muscarinic agonists from inhibiting cyclic AMP changes in neuroblastoma cells. When the phosphodiesterase inhibitor, MIX, was omitted from the assay system used here, smaller increases in cyclic GMP (1–2 fold) were obtained but all the agonists tested (except pilocarpine) caused similar increases (Table 1).

The explanation for this distinct difference in agonist response patterns in the presence and absence of a phosphodiesterase inhibitor is not clear. Basal levels of cyclic nucleotides will probably be different in the two states; if cyclic GMP act as a modulator of the effects of muscarinic agonists (see for example, Shultz, Schultz & Shultz, 1977) then the altered basal cyclic GMP level might change the overall response to muscarinic agonists and could explain the above results.

I wish to thank Mr. Peter Golds for technical assistance and the Smith, Kline and French Foundation for financial support.

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## Extracellular and intracellular recording during micro-iontophoresis: an appraisal

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The neuronal response produced by extracellular iontophoresis of many substances has been assessed either by extracellular recording of changes in neuronal firing and ventral root field potentials (VRF) or by intracellular recording of changes of membrane potential and conductance. Unfortunately, interpretation of results obtained by the two techniques can be contradictory (see McLennan, 1978), possibly because membrane properties are altered by penetration of a cell by a micro-electrode. We have investigated the relationship between the responses of motoneurones to extracellular iontophoresis of agents from a fixed electrode when the recording electrode was first positioned extracellularly and then moved intracellularly.

Cats were anaesthetised with pentobarbitone or chloralose and pentobarbitone. A circular seven barrelled iontophoretic unit was used, through the centre of which a screened recording electrode could be independently moved (see Spehlmann, 1969). Extracellular potentials were recorded through one of the barrels of the iontophoretic unit (1 M NaCl). The screen of the centre electrode could be driven either by its own signal or by the signal from the 'extracellular' barrel.

L-glutamate (1 M, pH 8), DL-homocysteate (DLH 0.3 M, pH 8), (−)-noradrenaline (0.2 M, pH 6) and NaCl (1 M) were applied iontophoretically. The electrodes were tracked through the $L_7$ and $S_1$ segments of the spinal cord (centre electrode protruding by 0–16 μm). After locating a neurone, the VRF and firing were recorded alternately through the 'extracellular' and centre electrodes before and during current balanced applications of DLH, glutamate and norad-

renaline. The centre electrode was then advanced (<60 μm) to penetrate the cell. Following successful impalement, the intracellular *and extracellular* activity was again recorded in response to the agents.

Iontophoretic application of DLH and glutamate reduced the VRF and caused a late positive wave. Cell firing could be evoked by both agents, that to glutamate being less marked and of shorter duration than that to DLH. The VRF did not change, or increased slightly in response to noradrenaline. The pattern of firing evoked by the amino acids was not significantly different before and after motoneurone penetration. Extracellular recordings during the depolarization and firing caused by DLH and glutamate showed that the negative wave of the VRF was reduced and the typical late positive wave appeared. Noradrenaline typically caused a hyperpolarization and inhibited the depolarizations and firing evoked by DLH and glutamate.

We conclude that the act of cell penetration does not in itself qualitatively alter the response of motoneurones to extracellular iontophoresis of DLH and glutamate and that these agents *decrease* and 'reverse' the VRF when the cells are depolarized.

Thus an increase of VRF during biogenic amine iontophoresis (Barasi & Roberts, 1977) does not necessarily indicate a neuronal depolarization but rather a hyperpolarization with action potential increase (Engberg, Flatman & Kadzielawa, 1976).

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## Pharmacological study of the anococcygeus muscle of the dog

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Pharmacological study of the anococcygeus muscle of rat and cat have been carried out (Gillespie, 1972; Gillespie & McGrath, 1974). In the present experiments the anococcygeus muscle of mongrel dogs of both sexes, weighing 2–6 kg, were used. The muscle was dissected out as described for rat anococcygeus muscle (Gillespie, 1972) and suspended in oxygenated Tyrode solution (37°C). The muscle showed no spontaneous contraction. Field stimulation of the muscle (0.5 ms, supramaximal voltage) produced frequency dependent motor responses which were blocked by guanethidine ($4 \times 10^{-6}$ M) and phentolamine ($3 \times 10^{-7}$ M) but unaffected by hexamethonium, atropine, promethazine or methysergide. When the tonus of the muscle was increased by guanethidine ($1 \times 10^{-4}$ M) or carbamylcholine ($3 \times 10^{-5}$ M); field stimulation caused the muscle to relax. The degree of relaxation was frequency dependent and the responses were not blocked by propranolol ($3 \times 10^{-6}$ M). Minimal and maximal contractions of the muscle were achieved by noradrenaline ($10^{-7}$ to $10^{-5}$ M), tyramine ($10^{-6}$ to $10^{-4}$ M), acetylcholine ($10^{-6}$ to $10^{-4}$ M), 5-hydroxytryptamine ($10^{-8}$ to $10^{-6}$ M), histamine ($10^{-7}$ to $10^{-4}$ M) and by higher doses of isoprenaline ($10^{-6}$ to $10^{-4}$ M). The effects of noradrenaline, tyramine and isoprenaline were blocked by phentolamine. The effects of acetylcholine, 5-hydroxytryptamine and histamine were blocked by atropine, methysergide and promethazine (but not cimetidine) respectively. Lower doses of isoprenaline ($10^{-9}$ to $10^{-7}$ M) relaxed the muscle when the tonus of the muscle was raised by guanethidine. The results suggest that the response of the anococcygeus muscle of the dog to field stimulation and to pharmacological agents is similar to the responses of the anococcygeus muscle of the rat except that in the dog the muscle has also $H_1$ receptors and beta adrenoceptors.

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