existing single methods in that it can monitor active metabolites of β-blockers that may contribute to the overall efficacy of the parent drug. Moreover, apart from being very sensitive and simple to perform, its use is not limited to a single β-blocking drug and application of the technique for routine plasma measurements can be anticipated.

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Effect of a phosphodiesterase inhibitor on cyclic GMP changes induced by muscarinic agonists in mouse neuroblastoma cells

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The potent muscarinic agonist, carbamoylcholine has been shown to elevate guanosine 3′:5′ phosphate (cyclic GMP) levels in mouse neuroblastoma cells, clone NIE 115 (Matsuzawa & Nirenberg, 1975; Strange, Birdsall & Burgen, 1977; Richelson, 1977). The response was inhibited by muscarinic antagonists but not by nicotinic antagonists. A phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine (MIX) was included in the assay system in order to obtain large (4–10 fold) and reproducible increases in cyclic GMP levels. These experiments have now been extended to include other muscarinic agonists (Table 1). Whereas agonist efficacy measurements indicated that a gradation of responses might have been expected, the agonists tested fell into two classes: potent agonists

Table 1 Effect of muscarinic agonists on cyclic GMP levels

Agonist	*Efficacy**	*Elevation of cyclic GMP over basal level produced by muscarinic agonists (10^{-3}M) expressed as a percentage of the elevation produced by carbamoylcholine (10^{-3}M)*	
		with MIX	*without MIX*
oxotremorine-M	N.D.	72	108**
carbamoylcholine	270	100**	100**
(+)-acetyl-β-methylcholine	107	88	N.D.
acetylcholine	71	107	N.D.
arecoline	32	2†	95**
oxotremorine	9	5	101**
pilocarpine	2.5	3	8†

Cyclic GMP was determined by a prelabelling method using [^{3}H]-guanine. Cyclic GMP elevations were measured by comparing duplicate or quadruplicate samples from cells stimulated with an agonist (applied for 60 s (with MIX) or 30 s (without MIX)) with duplicate or quadruplicate control samples (for basal cyclic GMP levels). The significance of the increase in cyclic GMP over the basal level was assessed where possible using a Wilcoxon test: ** significant at 5% level, † not significant at 5% level. The increase in cyclic GMP produced by agonists was expressed as a percentage of the increase produced by carbamoylcholine. N.D.—not determined. * N.J.M. Birdsall—unpublished results.

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.

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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-