opened and the mucosal surface carefully washed with water and blotted. The segments were then immersed in liquid nitrogen, weighed and homogenized in ice-cold acid butanol. Noradrenaline (NA), adrenaline (AD), dopamine (DA), and 5-hydroxytryptamine (5-HT) were extracted, and estimated fluorimetrically after trihydroxyindole formation (Ansell & Beeson, 1968, with the following modification by I.M. Martin, M.R.C. Neuropharmacology Research Unit, Medical School, Birmingham; personal communication). The supernatant from each homogenate was extracted with 10 ml iso-octane and 5 ml water. The aqueous phase (4.5 ml) was removed by a pipette pushed through the upper layer; no material at the interface was removed. This sample was processed as in the original method, but catecholamines were eluted from alumina with phosphate buffer pH 6.5 instead of acetic acid. Trihydroxyindoles were formed using 0.1 ml iodine reagent, 0.2 ml alkaline sulphate and 0.2 ml 6M acetic acid instead of the original quantities. AD was measured by Chang's (1964) method.

5-HT levels decreased aborally whereas NA levels increased (linear correlation coefficient, $P < 0.01$ and 0.001 respectively). DA and AD did not change significantly ($P > 0.1$) (Figure 1). 5-HT stimulates peristalsis in guinea-pig small intestine (Bülbring & Lin, 1958) whereas NA inhibits motility. Although the source of the amines (muscles, mucosa, blood vessels and nerves) is not known, these results are consistent with the finding that intestinal propulsion is more rapid proximally than distally.

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Investigation of the effects of drugs on morphine-induced contractions of the isolated colon of the rat

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Morphine causes some smooth muscle-containing tissue to contract (Weinstock, 1971). Whether or not morphine causes contractions depends on the dose, species and tissue examined (Vaughan Williams, 1954). In dog intestine, morphine produces contractions indirectly by releasing 5-hydroxytryptamine (5-HT) and acetylcholine (ACh) (Burks, 1973). This study sought to determine whether morphine had a similar effect in rat intestine. The effects of morphine were investigated by adding the drug to an organ bath containing 3–4 cm lengths of terminal colon suspended in oxygenated Krebs bicarbonate solution at 37°C. Responses of the colon were recorded isometrically.

Morphine (10^{-5} M) produced an immediate contraction followed at approximately minute intervals by waves of contractions, which gradually decreased in amplitude. When the tissue had ceased responding to the first dose of morphine, addition of a second dose was less effective in causing contractions. Prior addition of naloxone (5×10^{-6} M) to the bath prevented morphine producing contractions but naloxone (5×10^{-6} M) added after rhythmic activity had been initiated by morphine, was less effective in inhibiting the contractions. Morphine-induced contractions were not inhibited by atropine (10^{-4} M), hexamethonium (10^{-4} M) or tubocurarine (10^{-4} M). Indeed, these drugs occasionally potentiated morphine-induced contractions. Unlike in dog intestine, the responses produced by morphine in rat colon were dissimilar from those produced by ACh or 5-HT. The effects of 5-HT antagonists on the morphine-induced contractions were complex. Thus, lysergic acid diethylamide (3×10^{-7} M) did not affect

the morphine-induced contractions but when administered alone, caused contractions similar to those produced by morphine. In contrast, cyproheptadine (3×10^{-5} M) not only abolished morphine-induced contractions but also inhibited responses to carbachol (10^{-7} M) and potassium (30 mM).

Morphine-induced contractions were potentiated by phentolamine (10^{-5} M) and inhibited by noradrenaline (5×10^{-7} M) or tyramine (3×10^{-5} M). The dose of morphine required to produce contractions was lower in tissue from rats pretreated with reserpine (0.3 mg/kg daily for three days). In these tissues in which the frequency of spontaneous contraction was increased, tyramine (10^{-5} M) did not inhibit morphine-induced contractions.

Morphine-induced contractions were inhibited by adenosine (5×10^{-6} M), adenosinetriphosphate (ATP) (5×10^{-6} M), papaverine (5×10^{-5} M) and by removing calcium from the bathing medium, but were unaffected by tetrodotoxin (3×10^{-7} g/ml), which caused contractions similar to those produced by morphine.

These results suggest the mechanism underlying the actions of morphine in rat colon is unlike that responsible for the actions of morphine in dog intestine (Burks, 1973). They suggest that morphine may cause contractions in rat colon by removing a tonic inhibitory nervous influence, which normally suppresses myogenic activity involving a pacemaker (Connor, Prosser & Weems, 1974) within smooth muscle (Wood, 1972; Tonini, Leccinini & Crema, 1974).

M.G.C.G. is an M.R.C. Scholar.

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Increased cerebral cyclic GMP concentration induced by muscarinic cholinergic agonists and prostaglandin $F_{2\alpha}$

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An important role for cyclic nucleotides in the functioning of the central nervous system has been indicated by a number of investigations (see Daly, 1975). In previous studies in this laboratory the use of the neonate chick with its immature blood-brain barrier has allowed the investigation of the effects of several neurohormones on cerebral cyclic AMP formation *in vivo* (Edwards, Nahorski & Rogers, 1974). In view of the growing evidence that cyclic GMP may also be involved in the processes of neurotransmission (Goldberg, O'Dea & Maddox, 1973), we have made appropriate studies on the nucleotide using the above experimental model.

All experiments were performed on 3-day old male Ranger chicks. Drugs were injected into the right jugular vein and at appropriate intervals the cerebral hemispheres were removed by a freeze-blowing apparatus (Veech, Harris, Veloso & Veech, 1973). Cyclic GMP was assayed, after chromatographic purification with alumina and Dowex 50 resin, by a modification of the protein binding method of Dinnendahl (1974).

Unlike cyclic AMP (Nahorski, Rees & Rogers, 1975) the concentration of cyclic GMP (50–70 pmol/g) in the cerebral hemispheres was not influenced by decapitation or by age over the perinatal period. The administration of oxotremorine, arecoline or methacholine (0.1–0.5 μmol/kg) induced a rapid increase in cyclic GMP concentration. Maximum increases (100–200%) were observed 3 min after injection. Of a number of other substances studied prostaglandin $F_{2\alpha}$ (0.5 μmol/kg was also effective in increasing (80–100%) the nucleotide concentration *in vivo*. However, noradrenaline, dopamine, 5-hydroxytryptamine, adrenaline, isoprenaline, clonidine, histamine, γ-aminobutyric acid and prostaglandin E_1 were found to be ineffective, in doses up to those producing marked behavioural effects.