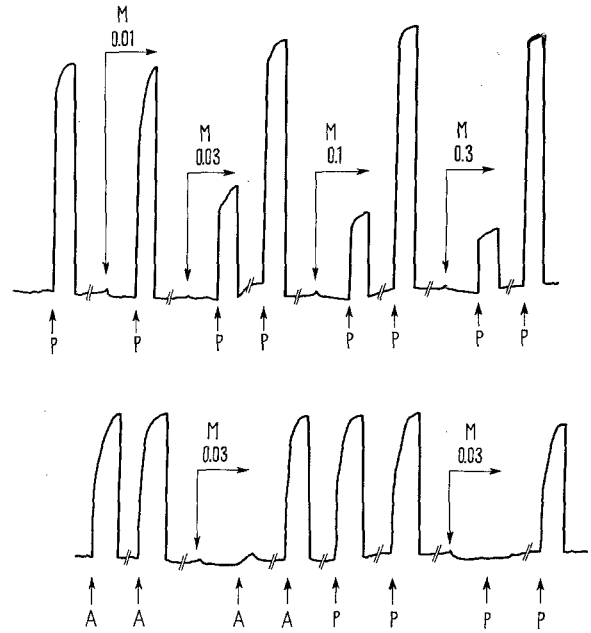
Morphine as Inhibitor of Prostaglandin E_1 in the Isolated Guinea-Pig Intestine

The prostaglandins are among the most powerful endogenous stimulants or inhibitors of the intestinal muscle known so far[1-8]. They have been found in the gastro-intestinal wall of man and many other species[1,9,10] and are formed in, or released from, various intestinal preparations[1,9]. Recently, the modes of action of prostaglandins E_1 and E_2 (PGE_1, PGE_2) on longitudinal and circular muscle layers of the human, guinea-pig and rat small intestine have been analyzed by BENNETT et al.[1]. On the basis of their rather extensive pharmacological analysis, these authors concluded that the prostaglandins contract guinea-pig and to some extent also human longitudinal muscle by a dual mode of action: (a) via stimulation of the intrinsic cholinergic nerves, and (b) by a direct action on or in the muscle cells.

MISIEWICZ et al.[11], on the other hand, just reported that PGE_1 administered orally in low dosage, apart from increasing transit-rate through the small intestine and the colon, is capable of causing abdominal colic and watery stools in healthy volunteers. This latter finding is of interest inasmuch as pronounced diarrhoea is also regularly observed in guinea-pigs given comparatively small doses of arachidonic acid (AA) or its peroxide (AAP) by stomach tubing[12]. The laxative action of AA or AAP in the guinea-pig has led us some time ago to examine such fatty acids with regard to their possible effect on the isolated small intestine of the guinea-pig. In fact, it was found that AA or AAP are capable of stimulating the isolated guinea-pig ileum at very low concentrations[13]. These contractions were easily inhibited, particularly by low concentrations of narcotic analgesics, e.g. morphine, etonitazene (*p*-ethoxybenzyl-2-dimethylaminoethyl-1-nitro-5-benzimidazole[14]) and by atropine at a higher concentration whereas pretreatment with cholinesterase inhibitors for instance potentiated them[13,15]. In view of some obvious similarities in the mode of action of both PGE_1 and AA in vivo and in vitro, we have examined the effect of morphine and other drugs on the contractions elicited by PGE_1 in the isolated guinea-pig ileum or jejunum. It will be shown that morphine acts as powerful antagonist of PGE_1 in the small intestine, an effect which has hitherto not been described despite the large amount of experimental data already at hand on the mode of action of prostaglandins.

The experimental procedures used were the same as those published previously[13,15]. Under these conditions, PGE_1 was found to cause contraction of the guinea-pig ileum or jejunum in concentrations as low as 0.1 to 0.3 ng/ml, i.e. a range somewhat lower than that described by BENNETT et al.[1] for the guinea-pig ileal muscle. When compared with PGE_1 on the same preparations, AAP caused contractions of the intestine at concentrations some 10–20 times higher. On the other hand, equi-effective doses of PGE_1 and AAP were equally well antagonized by morphine (see Figure). Another highly potent narcotic analgesic, etonitazene[14], proved an even more potent antagonist of PGE_1-induced contractions of the guinea-pig intestine, and the quantitative difference between these 2 analgesics with regard to contractions caused by PGE_1 was about the same as that observed previously on AAP-induced contractions[13]. As demonstrated in a previous paper on AAP, contractions elicited by PGE_1 proved more resistant to atropine than those caused by acetylcholine at equi-effective concentration. This finding is in accordance with that made by BENNETT et al.[1] using hyoscine. The effect of other drugs tested for antagonism to PGE_1 in the isolated guinea-pig intestine and their activities are shown in the Table. In the light of these data it is evident that, in addition to narcotic analgesics, a typical narcotic antagonist, nalorphine, is also capable of inhibiting PGE_1-induced contractions of the guinea-pig intestine whereas aminopyrine as a typical representative of the mild analgesics and anti-inflammatory drugs does not affect the prostaglandin effect in this organ. This finding is in contrast with other observations, in which aminopyrine was found to be an efficient antagonist of AAP-induced contractions[13,15]. On the other hand, as already mentioned briefly in another



Upper tracing: Isolated guinea-pig ileum. Inhibitory effect of morphine (M) at increasing concentrations (0.01–0.3 µg/ml) on contractions elicited by PGE_1 (P), 0.1 µg/ml. Morphine added 2 min prior to PGE_1. PGE_1 washed out after 1 min and re-added every 6–8 min. Lower tracing: Isolated guinea-pig jejunum. Inhibition of equi-effective doses of arachidonic acid peroxide (A), 0.3 µg/ml, and PGE_1 (P), 0.1 µg/ml, by morphine (M), 0.03 µg/ml.

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publication[16], the benzyl glucofuranoside, Glyvenol®, which is also an anti-inflammatory compound, exerts its prostaglandin-antagonistic action at about the same concentration at which it antagonizes histamine and other spasmogens in the guinea-pig intestine[16,17]. That this effect is not the result of an anti-histamine activity is revealed by the fact that tripelennamine (Pyribenzamine®) inhibits PGE_1 only at concentrations far in excess of those capable of antagonizing equi-effective histamine concentrations. From the evidence available so far it may be concluded that, in the guinea-pig intestine, PGE_1 possesses a mode of action practically identical with that of AAP described in 2 previous papers[13,15], the only difference being a quantitative one inasmuch as PGE_1 is the more potent stimulant of the two. On the other hand, the finding that morphine and similar compounds are powerful antagonists of the prostaglandin action in the intestine together with the observation that PGE_1 causes diarrhoea in man[11] may serve to explain the fact why opiates have been used in the treatment of diarrhoea.

In connection with some of the findings outlined above, still another point of quite a different and apparently unrelated interest arises. It is conceivable that AA, either directly or through biosynthetic transformation into PGE_2[3,4,7] possessing activities similar to those of PGE_1 in the small intestine[1], accelerates intestinal transit and/or causes diarrhoea in man in much the same way as it does in the guinea-pig[12], i.e. in a manner identical with that described for PGE_1 by MISIEWICZ et al.[11]. AA being an important ingredient of diets rich in unsaturated fatty acids that are claimed to possess lipid-lowering properties[18], one should consider the possibility that such diets may owe their hypolipidaemic action, at least partly, to a non-specific effect, namely accelerated intestinal transit[19].

Antagonistic action of some narcotic nd non-narcotic analgesics, anti-inflammatory drugs and other compounds on PGE_1-induced contractions of the isolated guinea-pig ileum or jejunum

Compound	ED_{50} (μg/ml $\pm$ S.E.)[a]
Morphine	0.01 $\pm$ 0.002
Etonitazene	0.000023 $\pm$ 0.000002
Nalorphine	0.014 $\pm$ 0.002
Aminopyrine	100[c]
C. 21,401-Ba[b] (Glyvenol®)	5.0 $\pm$ 0.40
Atropine	0.34 $\pm$ 0.04
Azamethonium	100[c]
Tripelennamine	6.2 $\pm$ 0.08
Fluoride (sodium)	30[c]
Cyanide (sodium)	22 $\pm$ 2.8

[a] $n = 4$. [b] Ethyl-3,5,6-tri-O-benzyl-glucofuranoside. [c] No effect observed at concentration shown.

Zusammenfassung. Es wird gezeigt, dass Analgetika vom Morphintypus die am isolierten Meerschweinchendünndarm nach Zugabe von Prostaglandin E_1 erfolgenden Kontraktionen ähnlich wie die durch Arachidonsäure (-peroxid) erzeugten Kontraktionen in spezifischer Weise zu antagonisieren vermögen.

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Amin-Transport hypothalamischer Vesikel

Subzelluläre, noradrenalinspeichernde Vesikel des Hypothalamus nehmen durch einen ATP-Mg^{++}-unabhängigen und einen ATP-Mg^{++}-abhängigen Mechanismus Noradrenalin auf[1]. Die ATP-Mg^{++}-unabhängige Aminaufnahme findet bei 0° und 37°, die ATP-Mg^{++}-abhängige Aufnahme dagegen nur bei 37 °C statt. Es wird deshalb angenommen, dass im Zentralnervensystem Noradrenalin sowohl passiv als auch durch einen aktiven, ATP-Mg^{++}-abhängigen Mechanismus in die subzellulären Partikel aufgenommen werden kann. In der vorliegenden Arbeit wurde die Abhängigkeit der beiden Prozesse von der Zeit sowie der Einfluss von Kalziumionen auf den Amininflux untersucht.

Methoden. Noradrenalinspeichernde Vesikel des Schweinehypothalamus wurden, wie früher beschrieben[2], durch Differentialzentrifugieren in 0,3 *M* Saccharose isoliert und in 0,17 *M* Histidin-HCl-Puffer pH 7,4 suspendiert. Die Inkubationsansätze enthielten 3 ml Vesikelsuspension, 0,5 ml Histindinpuffer pH 7,4, 0,5 ml $30 \times 10^{-3} M$ ATP, 0,5 ml $30 \times 10^{-3} M$ $MgCl_2$ oder $CaCl_2$ und 0,5 ml Amin (5×10^{-7} bzw. $3 \times 10^{-6} M$). Alle Substanzen wurden in Histindinpuffer gelöst. Die Ansätze wurden unter Schütteln (Frequenz: 100/min) 15 min lang inkubiert, zentrifugiert, die Rückstände mit 10 ml Histidinpuffer gewaschen, erneut zentrifugiert und mit 4 ml 0,4 *N* $HClO_4$ extrahiert[2]. Die Amine wurden im Überstand[3], das Eiweiss im Rückstand[4] bestimmt.

Versuche. Figur 1 zeigt die Zeitabhängigkeit der ATP-Mg^{++}-bedingten Noradrenalinaufnahme in die hypothalamischen Vesikel. Während in Abwesenheit von ATP und Magnesium der Noradrenalingehalt der Vesikel ständig abnimmt (Noradrenalinkonzentration im Inkubationsmedium: $5 \times 10^{-7} M$), verursacht die Zugabe von ATP-Mg^{++} eine starke Zunahme des Noradrenalingehaltes. Bei 37 °C findet sowohl Freisetzung des endogenen als auch Aufnahme des zugegebenen Noradrenalins zum grössten Teil innerhalb der ersten 6 min statt.

Zur Untersuchung der Zeitabhängigkeit der ATP-Mg^{++}-unabhängigen Noradrenalinaufnahme wurden hypothalamische Vesikel mit einer grösseren Noradrenalinkonzentration ($3 \times 10^{-6} M$) inkubiert. Bei dieser Aminkonzentra-

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