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MORPHINE-THEOPHYLLINE INTERACTION: ANTAGONISM OR FACILITATION?

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1 Morphine-theophylline interactions were investigated in both acute and narcotic-dependent preparations, *in vitro* and *in vivo*, using four different experimental models: LD_{50} doses of morphine and naloxone in the mouse; naloxone-induced contractions in the electrically-stimulated and opiate-dependent isolated ileum of the guinea-pig; naloxone-induced jumps in the mouse; and calcium uptake in synaptosomal preparations.

2 The LD_{50} of morphine was significantly increased by theophylline.

3 The lethal effect of theophylline was potentiated by pretreatment of the animals with naloxone.

4 Theophylline displayed protective effects in the inhibitory response to morphine and antagonism to the withdrawal response induced by naloxone in the electrically-stimulated isolated ileum of the guinea-pig.

5 The number of jumps induced by naloxone in morphine-dependent mice was significantly diminished by theophylline.

6 The inhibitory effect of morphine on the synaptosomal uptake of calcium was decreased by theophylline.

7 The effects of both morphine and theophylline on the cyclic nucleotides and the possible role of calcium in these actions are discussed.

Introduction

Cyclic nucleotides have been implicated in the mechanism of action of the opiates. Both inhibition and stimulation of adenyl cyclase have been reported under basal conditions as well as during enzyme activation (see refs in Clouet & Iwatsubo, 1976). Collier & Francis (1975) have suggested that cyclic adenosine 3′,5′-monophosphate (cyclic AMP) participates in the expression of the syndrome associated with morphine withdrawal. A large part of their evidence is based on the induction of the so-called 'quasi-morphine withdrawal syndrome' (QMWS), a state of psychomotor excitation induced by the administration of methylxanthines followed by naloxone, a narcotic antagonist. The intensity of the QMWS appears to be related to the level of phosphodiesterase inhibition (Butt, Collier, Cuthbert, Francis & Saeed, 1979).

However, data exist which contradict those already mentioned. Matsuda (1970) obtained an inhibition of the development of tolerance to morphine following the joint administration of the opiate and caffeine. Ho, Loh & Way (1973) observed antagonism to morphine-induced analgesia by cyclic AMP. Finally, Jhamandas, Sawynok & Sutak (1978) found antagonism to the inhibitory effect of morphine upon acetylcholine release by several methylxanthines.

Many of the differences between these results are due to variations in the doses and schedules of administration. We attempted to control some of these variables within the framework of a pharmacological model for the apnoea syndrome which is found during sleep in newborn infants. Endorphins might be implicated in this type of apnoea (Brailowsky, Luján & Shkurovich, 1978) since morphine is a well-known respiratory depressant and exogenously administered endorphins also have the same effect in experimental animals (Flórez & Mediavilla, 1977). Therefore, theophylline, which has long been successfully employed in the treatment of this disorder, and morphine interactions were studied.

The problem was approached as follows. Initially, dose-response (% deaths) curves were determined for morphine, theophylline, and naloxone, alone and

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in combination. Next, pharmacological models of proven efficacy in the experimental study of the opiates were used: the isolated guinea-pig ileum and the jump test in opiate-dependent mice (Ehrenpreis & Neidle, 1975). Finally, synaptosomal preparations were used to investigate the uptake of calcium, an ion necessary for adenyl cyclase and phosphodiesterase activity, enzymes responsible for the metabolism of cyclic AMP (see refs in Rasmussen & Goodman, 1977).

Methods

Taconic mice (20–30 g, 30–40 days old) that were bred in the School of Medicine's animal facilities were used in these studies. The *in vivo* experiments involved acute administration of drugs an the subsequent determination of the LD_{50}, as well as chronic exposure to morphine by pellet implantation and subsequent testing of the response to naloxone (jump test).

In vivo *experiments*

LD_{50} determination The animals were housed 10 to a cage at least 24 h before drug administration. Theophylline (10–300 mg/kg), morphine (50–400 mg/kg), and naloxone (3–1000 mg/kg) were injected intraperitoneally (i.p.), in 0.9% w/v NaCl solution (saline) or in the case of theophylline, in a slightly akaline aqueous solution (see Drug subsection) as a vehicle (10 ml/kg). The animals were immediately returned to their home cages and observed for 24 h. The LD_{50}s were determined by the method of Litchfield & Wilcoxon (1949). The LD_{50} was determined for morphine alone and following theophylline (10 mg/kg) injection immediately prior to morphine. The LD_{50} for theophylline was also obtained following the administration of a fixed amount of naloxone (10 mg/kg).

Jump test in morphine-dependent mice Animals housed 5 to a cage were injected subcutaneously with either theophylline (10 mg/kg) or vehicle 3 times a day (08 h 00 min, 14 h 00 min, 20 h 00 min) for 4 full days. A pellet containing either placebo or morphine (75 mg morphine base) was implanted subcutaneously on day 2 (08 h 00 min). The last injection of xanthine on day 5 (08 h 00 min) was made 2 h before naloxone (0.03–3 mg/kg) challenge (10 h 00 min). Immediately after the (i.p.) injection of the opiate antagonist, the animals were placed individually in glass cyclinders (diameter, 10 cm; depth, 30 cm) and the number of jumps over the period of an hour were recorded. A jump was defined as the simultaneous lifting of the four paws from the horizontal surface.

In vitro *experiments*

The 'acute' actions of morphine and its interaction with theophylline were studied *in vitro* in the guinea-pig isolated ileum by the method of Paton (1957), while the 'chronic' effects of morphine (physical dependence) were investigated in the same preparation according to the method of Rodríguez, Luján & Vargas-Ortega (1980). Briefly, male guinea-pigs (300–600 g) were killed by a sharp blow on the head and the small intestine was rapidly removed. The 10 cm nearest to the ileo-caecal junction was discarded and the resulting terminal section was used. Segments (1.5–3 cm) were set up in a 10 ml bath and were perfused with Krebs bicarbonate solution containing (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2 PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.0 and choline chloride 0.03 that had been warmed to 37°C and bubbled with 95% O_2 and 5% CO_2. The resting tension was fixed at 1 g and the longitudinal contractions of the segments were isometrically recorded by means of a Grass FT-03 force displacement transducer on a Grass 7D polygraph. After 30–40 min of continuous perfusion with Krebs solution at a rate of 10 ml/min to allow the equilibration of the intestinal segments, the acute and chronic experiments were performed.

Acute effects of morphine and morphine plus theophylline in the electrically-stimulated guinea-pig ileum Once the preparation was stabilized, the ileum was coaxially stimulated via two silver electrodes; the anode was placed intraluminally. Rectangular current pulses of 0.5 ms duration and of sufficient voltage to produce maximal responses were applied to the tissue at a frequency of 0.1 Hz with a Grass S88 stimulator. After the maximal tension had been established, a dose-response (% inhibition of contraction) curve to morphine was constructed. Each dose of narcotic was added directly to the organ bath in 100 μl of distilled water. After the maximal inhibitory response was obtained, the opiate was rapidly removed by flushing the bath with Krebs solution for 10 min. The tissue was then allowed to recover for 10 min without perfusion before the next dose of morphine was tested. The same procedure was carried out with either theophylline (1×10^{-6} M) or vehicle added to the Krebs solution. The ilea were perfused with this solution for 30 min before the addition of morphine and during the construction of the dose-response curve.

Morphine dependence in the guinea-pig isolated ileum Immediately after the ileum reached equilibrium, the nonstimulated preparations were perfused for 240 min at a rate of 10 ml/min with Krebs solution containing morphine (1×10^{-6} M) alone or with theophylline (1×10^{-6} M). Another set of preparations

was perfused with morphine alone for 235 min and for the final 5 min with morphine plus theophylline. Once the 240 min period had elapsed, the perfusion was discontinued, various doses of naloxone were added to the bath, and the resulting peak tension was measured. Each segment was used for only one determination.

Calcium uptake in synaptosomal preparations

Synaptosomes were prepared according to the method of Cotman & Matthews (1971). Aliquots of the synaptosomal suspension were incubated at 30°C for 2 min in a Dubnoff shaker. The following drugs were added to the medium along with $^{45}Ca^{2+}$: medium alone (control), theophylline (1×10^{-5} M), morphine (1×10^{-6} M), theophylline plus morphine, and naloxone (1×10^{-7} M) plus morphine. The time course of calcium uptake (1 to 10 min) was measured according to the method of Guerrero-Muñoz, Guerrero, Way & Li (1979).

Drugs

Theophylline, morphine HCl, and naloxone HCl were obtained from Sigma, Merck, and Endo, respectively. The morphine pellets were a gift of Dr E.L. Way. All chemicals used in the Krebs solution were of reagent grade. All the drugs were dissolved in distilled water with the exception of theophylline which was dissolved in a minimal amount of 0.1 M NaOH, partially neutralized with HCl, and diluted to the desired volume with distilled water at the final pH of 8.0.

Statistics

The method of Litchfield & Wilcoxon (1949) was used to determine the LD_{50} within 95% confidence limits (CL 95). The parallel line assay of Finney was used to assess the similarity of the curves and Student's *t* test was used to compare the effects of the various combinations of drugs.

Results

Figure 1 shows the dose-response (% deaths) curves for theophylline and morphine injected (i.p.) alone and together. There was a significant displacement of the morphine-theophylline curve such that the LD_{50} (CL 95) had to be calculated by extrapolation. The dose of theophylline used (10 mg/kg) was chosen on the basis of the data included in Table 1 which gives the results of the dose-response curves for theophylline and naloxone, injected (i.p.) alone and together. Naloxone potentiated the lethal effects of theophylline. The animals were observed for 24 h; death followed a generalized convulsive state.

There is evidence that the effects attendant on the acute and chronic administration of narcotics are dependent on different mechanisms of action

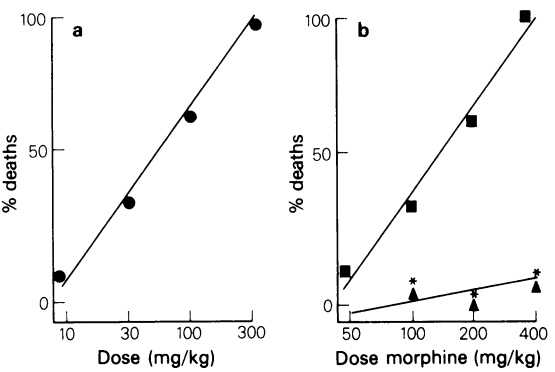

Figure 1 Lethal effects of (a) theophylline (●) and (b) morphine administered alone (■) or together (theophylline, 10 mg/kg) (▲). The following LD_{50}s were obtained by the method of Litchfield & Wilcoxon (1949): theophylline, 70 mg/kg (CL 95: 32–154); morphine, 148 mg/kg (102–197); theophylline plus morphine (extrapolated from curve), 1100 mg/kg (450–1500). $n = 20$ for each point. Comparisons between the effects of theophylline plus morphine and morphine alone at each dose of the opiate were made by Student's *t* test: * $P < 0.001$.

Table 1 Potentiation of the lethal effects of theophylline by naloxone in mice

Dose (mg/kg, i.p.)	*Naloxone*†	*Theophylline*†	*Naloxone* (10 mg/kg)§ *plus theophylline*†
3	0	5	10
10	0	10	40**
30	0	30	60*
100	15	70	100*
300	50	95	100*
1000	90	100	—

† Lethality is expressed in terms of % deaths ($n = 20$ at each dose). § Naloxone was injected immediately before the administration of the xanthine. The combined effect of naloxone (10 mg/kg) plus theophylline was compared with that of theophylline alone using Student's *t* test: * $P < 0.05$; ** $P < 0.01$.

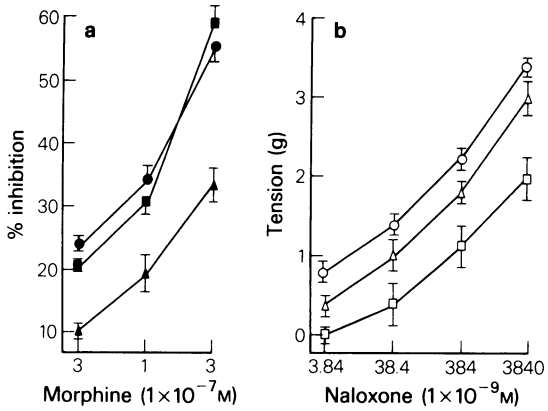

Figure 2 Acute (a) and chronic (b) effects of morphine and theophylline in the guinea-pig ileum. (a) % inhibition of the response (contraction) elicited by electrical stimulation under the following conditions: before (■) and after 30 min perfusion with Krebs solution containing the vehicle (see Methods, ●) or theophylline (1×10^{-6} M, ▲) prior to the dose-response curve with morphine. Perfusion with Krebs plus vehicle or theophylline was continued during the addition of morphine. A dose-response curve was obtained with each intestinal segment (n = 10, see Methods). (b) Strength of the contraction (expressed in g of tension) constituting a withdrawal response following naloxone administration after 240 min of exposure to morphine (1×10^{-6} M) under the following conditions: morphine alone (△); morphine plus theophylline (1×10^{-6} M) incubated together for 240 min (○); morphine plus theophylline (1×10^{-6} M) in which the tissue was incubated alone with morphine for 235 min and theophylline added for the last 5 min before naloxone administration (□). Note that the withdrawal response to naloxone was significantly diminished only under the latter condition. Each segment was used only once. n = 10 per point. Results are expressed as the mean (points) ± s.e. mean (vertical lines).

(Rodríguez & Villareal, 1974; Rodríguez *et al.*, 1980); thus, both situations were studied. The effects of theophylline on the inhibitory response elicited by the acute administration of morphine in the electrically-stimulated guinea-pig ileum are shown in Figure 2a. The dose-response (% inhibition) curves for morphine in tissues before and after 30 min of incubation with vehicle or theophylline (1×10^{-6} M) demonstrate that theophylline significantly reduces the effect of morphine. The influence of theophylline on naloxone-induced contractions in guinea-pig ilea that had been chronically exposed to morphine (1×10^{-6} M, 240 min) is depicted in Figure 2b. Theophylline (1×10^{-6} M) was added at the same time as morphine (for 240 min) or only 5 min before naloxone was added (after 235 min incubation with the opiate); only in the latter case was antagonism (4.8 times below that of morphine; CL 95, 2.1–10.9) to the withdrawal response observed. However, in the case of concurrent administration, there was a potentiation (2.2 times above that of morphine; C.L. 95, 1.1–5.6) of the withdrawal response.

The effects of theophylline on the responses of morphine-dependent mice in the jump test are shown in Table 2. No behavioural changes were observed in the animals after the administration of drugs before naloxone challenge. Theophylline significantly protected the morphine-dependent animals from the withdrawal effects elicited by naloxone.

Finally, the morphine-theophylline interaction was explored at the molecular level using mouse synaptosomes. Figure 3 shows the effects of morphine and theophylline on calcium uptake. When these two drugs were stimultaneously incubated with synaptosomes, the effect of morphine (decreased calcium uptake) was inhibited by theophylline.

Table 2 Effect of theophylline on the response of morphine-dependent mice to naloxone as measured by the jump test

Naloxone (mg/kg)	*Vehicle plus placebo*† (A)	*Vehicle plus morphine*† (B)	*Theophylline plus morphine*† (C)	*Theophylline plus placebo*† (D)
0.03	0	50 ± 12	6 ± 3*	2.0 ± 0.9
0.1	0	120 ± 16	22 ± 14*	3.6 ± 1.2
0.3	3.0 ± 1.8	186 ± 10	64 ± 20*	6.0 ± 2.3
1.0	6.0 ± 2.2	254 ± 18	82 ± 10*	8.4 ± 1.3
3.0	14.0 ± 3.2	224 ± 18	88 ± 10*	16.2 ± 4.6

Theophylline (10 mg/kg) or vehicle (see Methods) was injected subcutaneously 3 times a day (08 h 00 min, 14 h 00 min, 20 h 00 min) for 4 full days. Pellets containing either morphine or placebo were implanted on day 2 (08 h 00 min), naloxone was injected i.p. on day 5, 2 h (10 h 00 min) after the last theophylline injection (08 h 00 min), and the number of jumps was immediately recorded for 1 h. † The data are expressed as the mean number of jumps per hour ± s.e. mean (n = 20 per group). The effect of the various treatments were compared by Student's t test: * $P < 0.001$ for (A) and (B) vs. (C) at each naloxone dose tested; P is not significant for (A) vs (D).

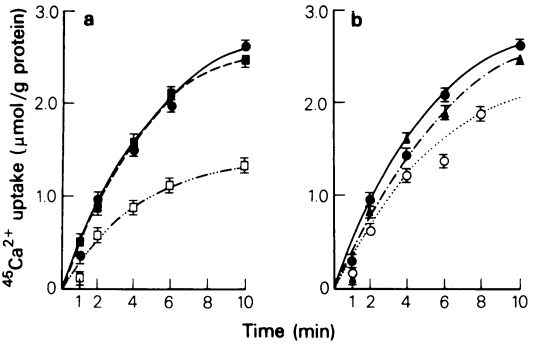

Figure 3 Effects of theophylline and morphine on the synaptosomal uptake of calcium. The time course of $^{45}C^{2+}$ uptake (μmol/g protein) was measured following incubation with: (a), medium alone (control, ●); morphine (1×10^{-6} M) (□); naloxone (1×10^{-7} M) plus morphine (1×10^{-6} M) (■); and (b), medium alone (control, ●); theophylline (1×10^{-5} M) (○); theophylline (1×10^{-5} M) plus morphine (1×10^{-6} M) (▲). Theophylline alone produced a nonsignificant decrease in calcium uptake but significantly blocked the inhibitory effect of morphine. Naloxone was also an antagonist of the morphine-induced inhibition of uptake. Each point represents the mean ± s.e. mean of three experiments each carried out in triplicate.

Discussion

Theophylline has been found to have three types of effects in this study: (a) Antagonism to the effects of morphine in: the lethality of the alkaloid (Figure 1); the acute inhibition of electrically-induced contractions in the guinea-pig ileum (Figure 2a); the intensity of the responses of dependent ilea to withdrawal induced by naloxone administration (although only when the theophylline is acutely incubated with the tissue) (Figure 2b); the jump test using mice (Table 2); and the inhibition of synaptosomal uptake of calcium (Figure 3a). (b) Facilitation of the effects of morphine in the dependent guinea-pig ileum when theophylline is chronically administered (Figure 2b), and on calcium uptake by synaptosomes when theophylline is administered alone (Figure 3a). (c) Lethality of theophylline was potentiated following pretreatment with naloxone (Table 1).

The antagonistic effects of theophylline are predominantly observed when it is administered acutely, the only exception being in the jump test (Table 2). The protection exercised by theophylline in these cases could be due to an increase in the intracellular concentration of calcium and/or cyclic AMP. The former could arise from the mobilization of the cation found in intracellular deposits or by a decrease in the enzymatic utilization of calcium by phosphodiesterase, while the latter could occur following the blockade of cyclic AMP destruction. There is evidence that both possibilities can bring about an inhibition of the effects of morphine (Sanghvi & Gershon, 1977; Sawynok & Jhamandas, 1979).

The inhibition of the opiate effect by theophylline which we found in the jump test contrasts with the results of other investigators. Collier & Francis (1975) have suggested that there is an association between the signs of withdrawal and an increase in cyclic AMP concentration when theophylline is administered orally (100 mg/kg). This dose produced a 70% mortality rate in our mice (Table 1). Ho, Loh, Bhargava & Way (1975) also found an increase in the development of tolerance and dependence when theophylline (100 mg/kg, i.p.) or cyclic AMP (i.v.) was given to mice implanted with morphine pellets. However, in neither case were toxic effects commented upon. It should be mentioned that in the latter study, the systemic administration of cyclic AMP did not increase the nucleotide concentration in the cerebrospinal fluid, implying that it did not pass the blood-brain barrier (Sebens & Korf, 1975). Our results support other studies in which methylxanthines were found to antagonize certain narcotic effects; for example, Matsuda (1970) found that caffeine significantly decreased the tolerance developed by the repetitive administration of morphine, using dental pulp stimulation in rats to measure the effect. However, no important alterations in the state of opiate dependence itself were found.

Jhamandas *et al.* (1978) found antagonism to morphine-induced inhibition of acetylcholine release following the administration of theophylline as well as calcium (given intraventricularly). It has been found that the acute administration of opiates brings about a decrease in the calcium content of synaptosomes and inhibition of calcium uptake, while chronic treatment has opposite effects (Harris, Yamamoto, Loh & Way, 1977). These changes in calcium concentration, dependent on the steady state (narcotic-dependent or not) of the system, might be reflected in an initial slowing, followed by stimulation, of cyclic nucleotide metabolism after the chronic application of the opiate (see Sanghvi & Gershon, 1977). On the other hand, the facilitation of the chronic effect of morphine by the xanthine in the guinea-pig ileum supports the hypothesis of Collier's group that the naloxone-induced withdrawal syndrome is related more to cyclic AMP than to calcium.

Finally, the potentiation of the lethality of theophylline by naloxone (Table 1) was an unexpected and interesting finding. Up to now, naloxone has not been shown to have definite effects when administered alone. The possibility that certain unknown actions of this substance may be uncovered by means of other drugs is attractive.

Many problems remain; our work adds some more variables to the list, while attempting to call attention to the importance of variables associated with the administration of drugs—especially dose and

duration of treatment—as well as the type of preparations employed. It is wise to keep in mind that there are different opiate receptors as well as different agonists (e.g., see Wüster, Schulz & Herz, 1980), and that the role of calcium and the cyclic nucleotides has in no case been clearly defined.

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References

BRAILOWSKY, S., LUJÁN, M. SHKUROVICH, M. (1978). Hacia un modelo farmacológico del síndrome de apneas del sueño: resultados preliminaries. In *Programa General y Resumenes de las Comunicaciones. II. Congreso Nacional de Farmacología.* Guadalajara, Jalisco, March 8–11, Asociación Mexicana de Farmacología.

BUTT, N.M., COLLIER, H.O.J., CUTHBERT, N.J., FRANCIS, D.L. & SAEED, S.A. (1979). Mechanism of quasi-morphine withdrawal behaviour induced by methylxanthines. *Eur. J. Pharmac.*, **53**, 375–378.

CLOUET, D.H. & IWATSUBO, K. (1976). Opiates and the brain adenylate cyclase system. In *Tissue Responses to Addictive Drugs.* ed. Ford, D.H. & Clouet, D.H. pp. 297–313. Holliswood, New York: Spectrum Publications.

COLLIER, H.O.J. & FRANCIS, D.L. (1975). Morphine abstinence is associated with increased brain cyclic AMP. *Nature, Lond.*, **255**, 159–162.

COTMAN, C.W. & MATTHEWS, D.A. (1971). Synaptic plasma membranes from rat brain synaptosomes: isolation and partial characterization. *Biochim. biophys. Acta*, **249**, 380–394.

EHRENPREIS, S. & NEIDLE, A. (1975). *Methods in Narcotic Research.* New York: Marcel Dekker.

FLÓREZ, J. & MEDIAVILLA, A. (1977). Respiratory and cardiovascular effects of met-enkephalin applied to the ventral surface of the brain stem. *Brain Res.*, **138**, 585–590.

GUERRERO-MUÑOZ, F., GUERRERO, M.L., WAY, E.L. & LI, C.H. (1979). Effect of β-endorphin on calcium uptake in the brain. *Science, N.Y.*, **206**, 89–91.

HARRIS, R.A., YAMAMOTO, H., LOH, H.H. & WAY, E.L. (1977). Discrete changes in brain calcium with morphine analgesia, tolerance-dependence, and abstinence. *Life Sci., Oxford*, **20**, 510–505.

HO, I.K., LOH, H.H., BHARGAVA, H.N. & WAY, E.L. (1975). Effect of cyclic nucleotides and phosphodiesterase inhibition on morphine tolerance and physical dependence. *Life Sci., Oxford*, **16**, 1895–1900.

HO, I.K., LOH, H.H. & WAY, E.L. (1973). Cyclic adenosine monophosphate antagonism of morphine analgesia. *J. Pharmac. exp. Ther.*, **185**, 336–346.

JHAMANDAS, K., SAWYNOK, J. & STUART, M. (1978). Antagonism of morphine action on brain acetylcholine release by methylxanthines and calcium. *Eur. J. Pharmac.*, **49**, 309–312.

LITCHFIELD, J.T. & WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.*, **95**, 99–113.

MATSUDA, K. (1970). Experimental studies on the effective procedure to inhibit the development of tolerance to and dependence on morphine. *Arzneimittel-Forsch.*, **20**, 1596–1604.

PATON, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **11**, 119–127.

RASMUSSEN, H. & GOODMAN, D.B.P. (1977). Relationships between calcium and cyclic nucleotides in cell activation. *Physiol. Rev.*, **57**, 421–509.

RODRÍGUEZ, R., LUJÁN, M. & VARGAS-ORTEGA, E. (1980). Circannual variation in sensitivity of the guinea-pig isolated ileum to naloxone. *J. Pharm. Pharmac.*, **32**, 363–365.

RODRÍGUEZ, R. & VILLAREAL, J.E. (1974). Graded quantitation of morphine tolerance and dependence on the same physiological system: the mouse. In *Committee on Problems of Drug Dependence.* pp. 305–312. Washington, D.C.: National Academy of Sciences.

SANGHVI, I.S. & GERSHON, S. (1977). Brain calcium and morphine action. *Biochem. Pharmac.*, **26**, 1183–1185.

SAWYNOK, J. & JHAMANDAS, K. (1979). Interactions of methylxanthines, nonxanthine phosphodiesterase inhibitors, and calcium with morphine in the guinea pig myenteric plexus. *Can. J. Physiol. Pharmac.*, **57**, 853–859.

SEBENS, J.B. & KORF, J. (1975). Cyclic AMP in cerebrospinal fluid: accumulation following probenecid and biogenic amines. *Expl Neurol.*, **46**, 333–344.

WÜSTER, M., SCHULZ, R. & HERZ, A. (1980). The direction of opioid antagonists towards μ-, δ- and ε-receptors in the vas deferens of the mouse and the rat. *Life Sci., Oxford*, **27**, 163–170.

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