period varying from 10 to 25 s. However, ricinoleic acid 10 μg ml^{-1} introduced to the bath elicited increased contractions by the PGE_2 (Fig. 1), an effect totally reversible by washing out the test compound. Addition of ricinoleic acid, 10 μg ml^{-1}, to the Tyrode solution also produced a pronounced increase in the amplitude of the prostaglandin-evoked responses. But ricinoleic acid itself did not induce contractions in the concentration used.

The effect of ricinoleic acid on agonists other than PGE_2 was also examined. Ricinoleic acid, 10 μg ml^{-1} increased contractions induced by ACh, 20 ng ml^{-1}, and by histamine, 10 ng ml^{-1} (see Table 1).

Small amounts of indomethacin (2 μg ml^{-1}) added to the Tyrode solution prevented the potentiating effect of ricinoleic acid (Table 1). The intraperitoneal administration of indomethacin (10 mg kg^{-1}) also desensitized the guinea-pig ileum to the action of PGE_2 in presence of ricinoleic acid. However, contractions induced by PGE_2 alone were unaffected by indomethacin.

Our results indicate that ricinoleic acid is able to sensitize the ileal longitudinal muscle to the contractile effect induced by PGE_2, ACh and histamine but ricinoleic acid has a more pronounced effect on contractions to PGE_2 than on those of the other agonists. It has been suggested that ricinoleic acid stimulates the release of PG-like substances (Beubler & Juan 1979). That PG modulate the responses of the ileal smooth muscle to various agonists including ACh and histamine has been shown by Bennett et al (1975). An effect on intestinal concentration of PG/or a stimulation of PG-like release, or both, could explain those observations.

Furthermore, the observation that indomethacin, a PG synthesis inhibitor, in-vitro or in-vivo inhibits the potentiation of PGE_2, supports the concept that ricinoleate stimulates the synthesis of endogenous ileal prostaglandins, an action probably important for the cathartic properties of ricinoleic acid, the active constituent of castor oil.

REFERENCES

Bennett, A. (1978) in: Turner, P., Shand, D. G. (eds) Recent Advances in Clinical Pharmacology. Churchill Livingstone, Edinburgh, London & New York, pp 17–30

Bennett, A., Eley, K. G., Stockley, H. L. (1975) Prostaglandins 9: 377–384

Beubler, E., Juan, H. (1979) J. Pharm. Pharmacol. 31: 681–685

Collier, H. O. J. (1974) in: Robinson, H. J., Vane, J. R. (eds) Prostglandin Synthetase Inhibitors. Raven Press, New York, pp. 121–133

Karim, S. M. M., Amy, J. J. (1975) in: Karim, S. M. M. (ed.) Advances in Prostaglandins and Reproduction. Lancaster, pp 77–80

J. Pharm. Pharmacol. 1984, 36: 65–68
Communicated May 27, 1983

Possible role of prostaglandins in post-tetanic potentiation at the nerve-muscle junction in the longitudinal muscle strip of guinea-pig ileum

ONDŘEJ KADLEC*, KAREL MAŠEK, VLADIMÍR, RŮŽIČKA, ISIDOR ŠEFERNA, *Institutes of Pharmacology, Academy of Sciences and the Charles University Faculty of Paediatric Medicine, Albertov 4, 128 00 Prague, Czechoslovakia*

The effect of tetanic stimulation on the twitch responses of the longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum to electrical stimulation was investigated in the presence of naloxone. Under this condition, or after the addition of PGE_2, twitch contractions were maximal and no potentiation of twitches following tetanus was observed. In the presence of indomethacin (1 μmol $litre^{-1}$) twitches were diminished and post-tetanic potentiation (PTP) was manifested. PTP was seen with indomethacin concentrations of 1 to 20 μmol $litre^{-1}$ or after simultaneous addition of diphloretin phosphate (16 μmol $litre^{-1}$). Thus it seems unlikely that the effect of prostaglandins released during tetanic stimulation would be of key importance for the manifestation of PTP. Rather it is thought that a decrease in the release of acetylcholine from motor nerve terminals, and consequently smaller twitches in the presence of indomethacin, offer favourable conditions for PTP.

* Correspondence.

The phenomenon of presynaptic post-tetanic potentiation (PTP), a transient increase in evoked transmitter release after tetanic stimulation, has been described at both nicotinic (for refs see MacIntosh & Collier 1976; Ginsborg & Jenkinson 1976) and muscarinic (Kadlec et al 1979) synapses. At the muscarinic synapse of the longitudinal muscle-myenteric plexus in the guinea-pig ileum either kind of post-tetanic inhibition (Puig et al 1978) or PTP (Kadlec et al 1982) is observed depending on the stimulus parameters during tetanus. PTP is more pronounced in the presence of naloxone and of low concentrations of indomethacin; naloxone prevents those effects of endogenous opiate ligands released during tetanus that are responsible for the major part of post-tetanic twitch inhibition (Puig et al 1978). The output of acetylcholine (ACh) and resulting twitch contractions might be modified by prostaglandin E

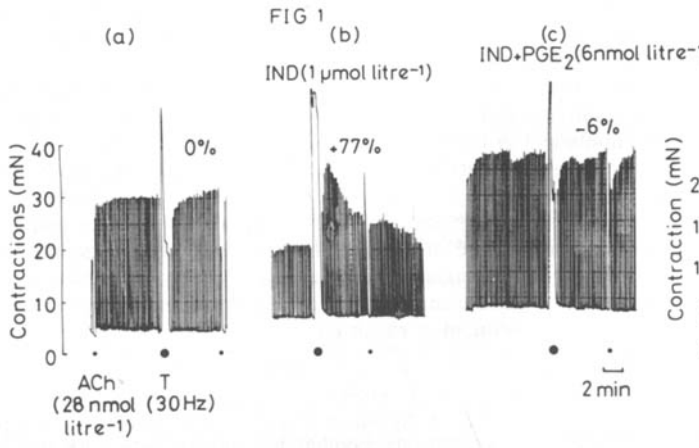

FIG. 1. The effect of indomethacin (IND 1 μmol litre^{-1}) and PGE_2 (6 nmol litre^{-1}) on the post-tetanic responses. ● (T) indicates tetanic stimulation (30 Hz, 50 s); (ACh) contraction evoked by ACh 28 nmol litre^{-1}. Changes in post-tetanic responses were 0, 77 and −6% respectively in (a) control in (b) the presence of indomethacin and in (c) the presence of indomethacin plus PGE_2. Naloxone (0·5 μmol litre^{-1}) was present throughout.

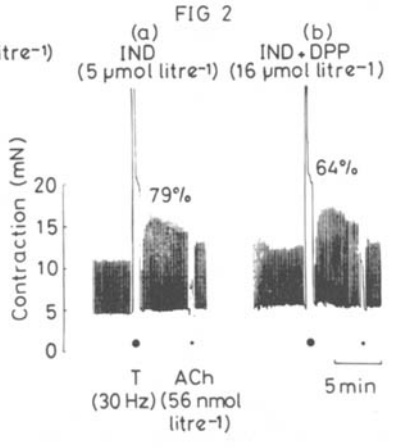

FIG. 2. The effect of DPP (16 μmol litre^{-1}) on the post-tetanic response in the presence of indomethacin (IND 5 μmol litre−1). ● (T) indicates tetanic stimulation (30 Hz, 50 s); (ACh) contraction evoked by acetylcholine 56 nmol litre^{-1}. Changes in post-tetanic responses were respectively 79 and 64% in the presence of indomethacin alone (a) and indomethacin plus DPP (b). Naloxone (0·5 μmol litre^{-1}) was present throughout.

(PGE) compounds and by indomethacin (Harry 1968; Kadlec et al 1974, 1978; Bennett et al 1975; Ehrenpreis et al 1976; Yagasaki et al 1981). Further, it has been shown that mechanical, electrical or neurotransmitter stimulation was followed by an increased release of tissue PGs (Piper & Vane 1971; Junstad & Wennmalm 1973, 1974; Kadlec & Mašek 1979). The aim of the present study was to elucidate whether a transient increase in the height of cholinergic twitches after tetanus (PTP) involved PG release.

Materials and methods

Male guinea-pigs (400–600 g) were killed by a blow on the head. Myenteric plexus-longitudinal muscle strips, 6 cm long, were prepared as described by Paton et al (1971), and mounted in a 4 ml organ bath under a load of 4 mN and allowed to equilibrate for 30 min. Changes in tension were recorded isometrically. The bathing medium (37 °C) was an aerated solution of the following composition (mmol litre^{-1}) NaCl 120, KCl 5·9, $CaCl_2$ 2·5, $NaHCO_3$ 15·4, $MgCl_2$ 1·2, $NaHPO_4$ 1·2, and glucose 11·5; also 0·5 μmol litre^{-1} naloxone. The preparation was stimulated by two platinum electrodes, one at either end of the organ bath. Rectangular pulses of 1 ms duration and supramaximal voltage (10–13 V cm^{-1}) at 0·1 Hz were used to evoke twitches, and 30 Hz produced tetanus. In the 5 min periods preceding or following tetanus the sums of twitch amplitudes (ΣpreT and ΣpostT, respectively) were calculated and the percent change induced by the tetanus evaluated: $[(\Sigma postT - \Sigma preT)/\Sigma preT] \times 100$. Thus positive values represent potentiation and negative values inhibition of twitches in post-tetanic period. A test dose of ACh (28 or 56 nmol litre^{-1} for 10 s) was applied 5 min before and after tetanic stimulation. The dose was chosen to give submaximal contractions, preferably with an amplitude comparable to that of twitches. The tetanic stimulation was repeated at 30 to 40 min intervals; indomethacin, PGE_2 or diphloretin phosphate (DPP) had been present for 30, 10 and 10 min respectively before their effects on PTP were tested.

Drugs used were: acetylcholine chloride (Germed), diphloretin phosphate (AB Leo), indomethacin (Sigma), naloxone hydrochloride (Narcan, Winthrop), prostaglandin E_2 (Upjohn). All drugs were dissolved in the physiological solution described above; indomethacin was first dissolved in sodium bicarbonate solution. Results are expressed as the mean ± s.e.m., with the number of experiments in parentheses. Student's two-tailed *t*-test for unpaired data was used for statistical analysis.

Results

The post-tetanic response was studied in the presence of naloxone; different concentrations of indomethacin and subsequently or separately DPP or PGE_2 were added (Table 1). The contractions induced by ACh were not significantly changed by any of these treatments, however, electrically evoked neurogenic twitches were significantly decreased in the presence of indomethacin alone or plus DPP; the inhibitory effect of indomethacin was reversed by the addition of PGE_2. In the presence of naloxone alone (control group; Fig. 1), or DPP alone, no significant PTP was observed. After the addition of indomethacin 1 μmol litre^{-1}, PTP was apparent and increased with increased indomethacin concentrations

Table 1. Effects of drugs on contractions and post-tetanic responses in myenteric plexus-longitudinal muscle preparations of the guinea-pig ileum. Naloxone (0·5 μmol litre^{-1}) was present throughout. Values are expressed as means ± s.e.m.; numbers of observations are given in parentheses.

Group (concn in μmol litre^{-1})	Contractions (mN) ACh (28 nmol litre^{-1})	Twitches (0·1 Hz)	Post-tetanic responses (%)
Control	20 ± 3 (23)	28 ± 3 (31)	5·5 ± 2·8 (13)
DPP 16	18 ± 2 (7)	22 ± 3 (7)	1·6 ± 11·6 (5)
Indomethacin 1	17 ± 2 (17)	15 ± 1** (21)	46·5 ± 9·5** (21)
IND 1 + PGE_2 6	27 ± 5 (4)	32 ± 1 (8)	−6·3 ± 10·5 (4)
IND 5	15 ± 2 (18)	7 ± 1** (20)	45·9 ± 11·1* (20)
IND 5 + DPP 16	14 ± 5 (6)	10 ± 3* (8)	28·7 ± 10·9* (6)
IND 20	12 (2)	7 (2)	31·0 (2)

* $P < 0·02$; ** $P < 0·005$, compared with the respective control group.

(Figs 1, 2). Also, the addition of DPP together with indomethacin did not significantly change the PTP (Fig. 2). Only the addition of PGE_2 together with indomethacin restored the post-tetanic response to the level observed in the control group without indomethacin (Fig. 1).

Discussion

In our previous work (Kadlec et al 1982) it was shown that PTP was due to changes in transmitter release as determined by ACh bioassay and [^{3}H]ACh output measurement. In the present study the drugs applied affecting the PG system did not significantly change ACh-evoked contractions. So, the observed effects of these concentrations of indomethacin at 1 and 5 μmol litre^{-1} and of PGE_2 on twitches might be primarily attributed to changes in transmitter release at the muscarinic synapse (Paton et al 1971). This confirms other reports where cholinergic twitches or the release of ACh from cholinergic motor nerve terminals were measured under the influence of drugs interfering with the PG system (Vane 1971; Northover 1971; Kadlec et al 1974, 1978; Bennett et al 1975; Ehrenpreis et al 1976; Yagasaki et al 1981). Further, the antagonism of the PG effect by DPP has been reported to be relatively selective, in the concentrations used, in decreasing the effects of PGE and PGF compounds compared with some other biologically active substances (Bennett & Posner 1971; Eakins et al 1973; Ishizawa & Miyazaki 1976). Thus the changes in neurogenic twitches and consequently the changes in the post-tetanic response might be attributed to the interference of indomethacin, DPP and exogenous PGE_2 with PG synthesis or action.

The highest concentration of indomethacin used (20 μmol litre^{-1}) should substantially reduce the synthesis of PGs even during any enhancement by tetanic stimulation (Ferreira et al 1972; Flower 1974; Ehrenpreis et al 1976); further, should any PGE or PGF be released despite the presence of indomethacin (5 μmol litre^{-1}) their effect would be expected to be diminished in the presence of DPP. Even 1 μmol litre^{-1} indomethacin should be sufficient for sustained inhibition of PG synthesis (Flower 1974). It therefore seems unlikely that PGs released during tetanus are necessary for PTP.

PTP was also observed in the absence of indomethacin when twitch height was decreased by noradrenaline or adenosine which act by a presynaptic mechanism (Kadlec et al 1982). However, these substances were less potent than indomethacin, and their inhibitory effect on twitches faded with time despite their continued presence. Thus the use of indomethacin with subsequent decrease in cholinergic twitch contractions is better for the demonstration of PTP at this muscarinic synapse.

We wish to thank Dr C. W. Stewart (Winthrop, U.K.) for the gift of naloxone hydrochloride (Narcane), Dr J. Pike (Uphohn Co., U.S.A.) for PGE_2, and Dr B. Högberg (Leo AB, Sweden) for diphloretin phosphate.

REFERENCES

Bennett, A., Eley, K. G., Stockley, H. L. (1975) Prostaglandins 9: 377–384

Bennett, A., Posner, J. (1971) Br. J. Pharmacol. 42: 584–595

Eakins, K. E., Fex, H., Fredholm, B., Högberg, B., Veige, S. (1973) Adv. Biosci. 9: 135–138

Ehrenpreis, S., Greenberg, J., Comaty, J. E. (1976) Eur. J. Pharmacol. 39: 331–340

Ferreira, S. H., Herman, A., Vane, J. R. (1972) Br. J. Pharmacol. 44: 328–330P

Flower, R. J. (1974) Pharmacol. Rev. 26: 33–67

Ginsborg, G. L., Jenkinson, D. H. (1976) in: Zaimis, E. (ed.) Neuromuscular Junction. Springer-Verlgag, Berlin, p 335

Harry, J. D. (1968) Br. J. Pharmacol. Chemother. 33: 213–214P

Ishizawa, M., Miyazaki, E. (1976) Prostaglandins 11: 829–840

Junstad, M., Wennmalm, A. (1973) Acta Physiol. Scand. 87: 573–574

Junstad, M., Wennmalm, A. (1974) Br. J. Pharmacol. 52:375–379

Kadlec, O., Mašek, K., Šeferna, I. (1974) Ibid. 51: 565–570

Kadlec, O., Mašek, K., Šferna, I. (1978) J. Pharmacol. Exp. Ther. 205: 635–645

Kadlec, O., Mašek, K., Šeferna, I. (1979) Eur. J. Pharmacol. 60: 383–386

Kadlec, O., Mašek, K. (1979) in: Tuček, S. (ed.) Cholinergic Synapse. Elsevier, Amsterdam, pp 203–209

Kadlec, O., Šeferna, I., Mašek, K., Somogyi, G. T., Knoll, J. (1982) Eur. J. Pharmacol. 79: 245–255

MacIntosh, F. C., Collier, B. (1976) in: Zaimis, E. (ed.) Neuromuscular Junction. Springer-Verlag, Berlin, p. 187

Northover, B. J. (1971) Br. J. Pharmacol. 41: 540–551

Paton, W. D. M., Vizi, E. S., Zar, M. A. (1971) J. Physiol. (London) 215: 819–848

Piper, P. J., Vane, J. R. (1971) Ann. N.Y. Acad. Sci. 180: 363–368

Puig, M.M., Gascón, P., Musacchio, J. M. (1978) J. Pharmacol. Exp. Ther. 206: 289–302

Vane, J. R. (1971) Nature (New Biol.) 231: 232–235

Yagasaki, O., Takai, M., Yanagiya, I. (1981) J. Pharm. Pharmacol. 33: 521–525

J. Pharm. Pharmacol. 1984, 36: 68–70
Communicated June 6, 1983

Evidence that drugs increasing 5-hydroxytryptamine transmission block jumping but not wet dog shakes in morphine-abstinent rats: a comparison with clonidine

S. ROMANDINI, L. CERVO, R. SAMANIN*, *Istituto di Ricerche Farmacologiche 'Mario Negri' Via Eritrea, 62-20157 Milano, Italy*

(+)-Fenfluramine, a 5-hydroxytryptamine (5-HT) releaser and uptake blocker, *m*-chlorophenylpiperazine (CPP), a 5-HT receptor agonist, and clonidine, an agonist at adrenoceptors, were studied for their ability to modify jumping and wet dog shakes in morphine abstinent rats. (+)-Fenfluramine and CPP blocked jumping with no effect on wet dog shakes whereas the reverse was true for clonidine. The results further show that 5-HT mechanisms are preferentially involved in the expression of jumping in morphine-abstinent rats.

Recent studies have shown that drugs which increase 5-hydroxytryptamine (5-HT) transmission inhibit naloxone-precipitated jumping in morphine-dependent rats, with little or no effect on other signs such as ptosis and diarrhoea (Samanin et al 1980; Cervo et al 1981).

Wet dog shakes, commonly studied as a withdrawal sign (Wei et al 1973; Lal & Numan 1975; Vetulani & Bednarczyk 1977), were not measured in these studies since they were present to a very limited extent in morphine abstinent rats. This may have been the result of the high level of morphine dependence used since it has been shown that the frequency of wet dog shakes is inversely related to the intensity of morphine dependence (Bläsig et al 1973).

The present study examined the effects of (+)-fenfluramine, a 5-HT releaser and uptake blocker (Garattini et al 1975) and *m*-chlorophenylpiperazine (CPP), a 5-HT receptor agonist (Samanin et al 1979), on wet dog shakes in two different naloxone-precipitated withdrawal syndromes where this sign is well represented. The effects were compared with that of clonidine, an agonist at adrenoceptors (Andén et al 1970), which has been found to inhibit wet dog shakes in rats (Tseng et al 1975) and some aspects of the morphine abstinence syndrome in man (Gold et al 1978).

* Correspondence.

Materials and methods

Male CD-COBS rats (Charles River, Italy) about 200 g at the beginning of the experiments, were housed (3 per cage) under constant room temperature (21 + 1 °C) and relative humidity (50%) with a 12 h light −12 h dark cycle (dark period commencing at 19.30). Food and water were freely available.

Induction of physical dependence. One group of rats (Exp. A) was made dependent by subcutaneous implantation of a pellet containing 75 mg morphine free base formulated according to the method of Gibson & Tingstad (1970). Withdrawal was tested 72 h after implantation. Another group of animals (Exp. B) received two intraperitoneal injections on day 1 (9 am and 6 pm) of 5 mg kg^{-1} morphine hydrochloride, calculated as free base. The dose of morphine was doubled every day thereafter to reach a total daily dose of 40mg kg^{-1} on day 4. At 9 am on day 5 the animals received the last injection of morphine (25 mg kg^{-1}) and were tested for withdrawal 4 h later.

Testing for withdrawal. The abstinence syndrome was precipitated in groups A and B with an intraperitoneal injection of 1·0 mg kg^{-1} naloxone HCl, dissolved in distilled water. Pellets in group A were not removed before testing. Before injection of naloxone, the animals were placed individually for 30 min acclimatization in test chambers consisting of rectangular Acryglass boxes (30 × 30 × 25 cm). Equal numbers of controls and experimental animals for each treatment were tested simultaneously.

Withdrawal signs within 30 min were recorded by observers uninformed of an animal's treatment conditions. Abstinence signs precipitated by naloxone in both experimental groups consisted mainly of wet dog shakes, diarrhoea, ptosis and, to a lesser extent, jumping. Other signs such as teeth chattering, vocaliza-