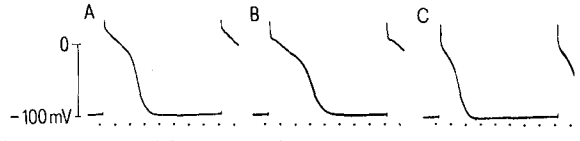
Purkinje fibres (personal observations). Electrical recording is achieved by standard microelectrode techniques[8].

Results and discussion. In a series of 26 experiments the ionophore shortened the action potential duration whether measured at 50%, 75%, 90% or 100% of repolarization. The ionophore decreased the level of the plateau whether measured 50, 100, 150 or 200 msec after onset of the action potential. In most experiments the ionophore hyperpolarized the membrane and the preparation became less easily excitable by an external stimulus. Exposure of the preparation to lanthanum chloride (50 μM), verapamil (1 mg/l), propranolol (10^{-6} M), or practolol (10^{-4} M) did not inhibit these effects of X-537A on the action potential.

Effect of X-537A on a canine Purkinje fibre preparation

	Overshoot (mV)	Plateau height[a] (mV)	Action potential duration[b] (msec)	Maximum diastolic potential (mV)
Control	+ 34	+ 2	322	— 94
Practolol	+ 27	— 5	424	— 93
X-537A	+ 23	— 31	209	— 95

[a] Level of the plateau with respect to 0 mV measured 100 msec after onset of the upstroke of the action potential. [b] Measured from the onset of the upstroke of the action potential to 100% repolarization.



The effect of X-537A on a Purkinje fibre.
A) Control, external stimulation at 1.2 Hz. B) After exposure to practolol 10^{-4} M for 137 min. C) After exposure to X-537A 10^{-5} M for 50 min. The external potassium concentration was 4 mM per liter. 0 mV and —100 mV are indicated by the vertical calibration. The horizontal dots are 100 msec apart.

The Figure shows the typical effect of X-537A (10^{-5} M) on a canine Purkinje fibre preparation that had been superfused for 137 min with practolol (10^{-4} M). Panel A) is the control action potential. Panel B) is the action potential after exposure to practolol (10^{-4} M) for 137 min. Panel C) shows the action potential after exposure to X-537A (10^{-5} M) for 50 min. The Table summarizes the result of this experiment.

The change in the overshoot (Figure and Table) may be due to an ionophore-induced increase in intracellular sodium concentration (Na_i) or the decrease in overshoot may be due to practolol exposure. The decrease in plateau potential is due to the increase in outward potassium current and to the decrease in inward driving force on calcium. These effects are due to the ionophore-induced increase in Ca_i. The dramatic shortening of the action potential duration results from the Ca_i-induced increase in potassium permeability. The slight ionophore-induced increase in maximum diastolic potential is not significant in this experiment but in other experiments performed with potassium concentrations of 2.7 mM or 2.0 mM, ionophore-induced increase in Ca_i caused significant hyperpolarization of the membrane.

In spontaneous preparations, X-537A suppressed spontaneous activity. The absence of calcium prevented the ionophore effect. Purkinje fibres of a dog pretreated with reserpine (total dose 1.2 mg/kg divided over 4 days) were exposed to practolol (10^{-4} M) and X-537A (10^{-5} M). The effect of X-537A on this preparation was identical to the effect on the preparation shown in the Figure.

These results tend to confirm the suggestion that Ca_i modulates potassium permeability in cardiac tissues[6,9] as it has been shown to do in other excitable cells[10,11].

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Role of 5-Hydroxytryptamine in Prostaglandin E_1-Induced Potentiation of Hexobarbitone Hypnosis in Albino Rats

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Summary. PGE_1 potentiated, while diclofenac, a prostaglandin synthesis inhibitor, antagonized hexobarbitone hypnosis in rats. PGE_1-induced potentiation of hexobarbitone sleep was inhibited by a 5HT synthesis inhibitor and by a 5HT receptor blocker, suggesting that this potentiation is 5HT mediated.

Prostaglandins are present in brain and other organs of mammals and are extremely active in a number of biological systems[2-4]. Prostaglandins of the E series have been shown to cause profound sedation, stupor and catatonia, when administered intraventricularly to cats or i.v. to chicks[5-7]. In a recent study[8], intraperitoneal administration of prostaglandin E_1 (PGE_1) was shown to produce marked sedation in rats, to the extent of producing a behavioural state superficially resembling normal sleep with, however, an EEG pattern seen in normal waking state. This PGE_1 induced sedation was accompa-

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Effect of prostaglandin E_1 and diclofenac on hexobarbitone hypnosis and effect of *p*-chlorophenylalanine and methysergide on prostaglandin E_1 induced potentiation of hexobarbitone hypnosis in albino rats

Groups		*n*	Sleeping time (min) Mean ± SE	*P*
1	Hexobarbitone	15	60.8 ± 4.15	—
2	Prostaglandin E_1 + hexobarbitone	10	89.0 ± 3.55	0.001 [a]
3	Diclofenac + hexobarbitone	10	43.7 ± 3.79	0.01 [a]
4	*p*-Chlorophenylalanine + prostaglandin E_1 + hexobarbitone	10	60.0 ± 7.72	0.01 [b]
5	Methysergide + prostaglandin E_1 + hexobarbitone	10	57.1 ± 7.35	0.01 [b]

[a] Significance in relation to control hexobarbitone group (Group 1). [b] Significance in relation to prostaglanding E_1 pretreated hexobarbitone group (Group 2).

nied by increased brain 5-hydroxytryptamine (5HT) turnover. PGE_1 has also been reported to potentiate hexobarbitone sleep in mice[9].

In a recent communication[10], we have reported that PGE_1 potentiates the antinociceptive effect of subanalgesic dose of morphine in rats, and this potentiation was shown to be 5HT mediated. We have also recently reported that hexobarbitone hypnosis in rats is 5HT-mediated[11]. It was therefore thought worthwhile to investigate the effect of PGE_1 on hexobarbitone sleep in rats and determine the role of 5HT in the interaction between these 2 drugs.

Material and method. Healthy Wistar albino rats, of either sex, weighing between 100–150 g were used. Food was withdrawn 18 h before and water immediately before experimentation. All experiments have been conducted at ambient temperature of 30 ± 2 °C. Hexobarbitone sodium (100 mg/kg i.p.) induced sleeping time was measured as the time interval between loss and regain of righting reflex. At least 10 animals were used for each control and drug pre-treated group. Student's *t*-test was used for statistical analysis.

The following drugs were used: hexobarbitone sodium (Bayer), PGE_1 (Upjohn), *p*-chlorophenylalanine (Sigma), methysergide (Sandoz) and diclofenac sodium (Ciba-Geigy).

PGE_1 (1 mg/kg) was administered 15 min before hexobarbitone. Diclofenac (15 mg/kg) was administered for 2 consecutive days, the second injection being given 4 h before hexobarbitone. *p*-Chlorophenylalanine (100 mg/kg) was administered for 3 consecutive days, the last injection being given 24 h before experimentation, while methysergide (5 mg/kg) was given 1 h before experimentation. All drugs used were administered i.p. and doses are expressed as salts.

Results. Results are summarized in the Table. PGE_1 significantly potentiated (46.3%) hexobarbitone sleeping time. Diclofenac, on the contrary, significantly inhibited (28.2%) hexobarbitone hypnosis. Pretreatment with *p*-chlorophenylalanine (*p*-CPA) or methysergide, significantly inhibited PGE_1 induced potentiation of hexobarbitone sleep by 32.6% and 35.96%, respectively.

Discussion. PGE_1-induced potentiation of hexobarbitone hypnosis in rats supports the earlier observation with mice[9]. This potentiation was found to be significantly inhibited by prior treatment with either a selective 5HT synthesis inhibitor, *p*-CPA[12], or a specific 5HT receptor antagonist, methysergide. This clearly implicates 5HT as the mediator in PGE_1 potentiation of hexobarbitone. PGE_1 has been reported to produce sedation in rats on i.p. administration, not by a direct effect, but through an increase in brain 5HT turnover[8]. Studies from this laboratory also indicate that PGE_1 enhances brain 5HT turnover in Wistar rats on i.p. administration[13].

It may be argued that the dose of PGE_1 used is relatively large and could lead to an unphysiological concentration of the drug in the brain. This point has been effectively countered in a recent communication[8], in which it was pointed out that 80% of injected PGE_1 is removed by the liver and 95% by the lungs[14] so that the concentration of PGE_1 actually reaching the brain after i.p. administration will be extremely small.

Considerable evidence links brain 5HT with sleep[15–18]. It thus appears possible that PGE_1, by increasing brain 5HT turnover, potentiates hexobarbitone-induced sleep, an event antogonized by drugs known to inhibit the synthesis or block the receptor activity of 5HT.

Diclofenac, a potent inhibitor of prostaglandin synthetase[19], significantly inhibited hexobarbitone sleep, suggesting that prostaglandins are somehow linked to hexobarbitone sleep.

In view of the observations of the present study, that inhibition of endogenous synthesis of prostaglandins antagonise hexobarbitone sleep and inhibition of 5HT synthesis or receptor action antagonizes PGE_1-induced potentiation of hexobarbitone sleep, coupled with the data that PGE_1 increases turnover of brain 5HT[8,13] and the reported 5HT mediation of hexobarbitone hypnosis in rats, it may be hypothetized that, in rats, hexobarbitone triggers off a PGE_1-induced increase in 5HT turnover, which in turn contributes to hexobarbitone sleep. However, this hypothesis requires further confirmatory data and work in this direction is in progress.

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