It is well known that 5-hydroxytryptamine in low concentrations contracts the guinea-pig ileum through intramural cholinergic nerve elements (M-receptors) (Rocha e Silva, Valle & Picarelli, 1953; Gaddum & Picarelli, 1957; Kosterlitz & Robinson, 1958; Brownlee & Johnson, 1963). Contraction of the ileum induced by 5-HT (5×10^{-7} M) was inhibited by morphine (10^{-6} M), leucine-enkephalin (10^{-6} M) and tetrodotoxin (3×10^{-7} M). Cimetidine (10^{-4} M) and naloxone (10^{-7} M), the concentrations which did not influence contraction of the ileum induced by 5-HT, abolished the inhibition by morphine and leucine-enkephalin (Fig. 2) but did not influence that by tetrodotoxin. The results thus suggest that cimetidine in high concentrations acts as a narcotic antagonist on the ileum from the guinea-pig.

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Enhancement of the abdominal constriction response of mice to lipopolysaccharides by phosphate

J. R. ROBORGH, *Philips-Duphar B.V., Research Laboratories, Postbox 2, Weesp, The Netherlands*

After intraperitoneal injection of a noxious agent into mice, they respond with repeated waves of constriction and elongation passing caudally along the abdomen ending with extension of the hind limbs. The reaction has been called the 'abdominal constriction response' (Collier, Hammond & others, 1964; Collier, Dinneen & others, 1968). In the course of an investigation of local reactions to parenteral preparations, this response of mice was used as a test model and a remarkable enhancement of the constriction response to bacterial lipopolysaccharides was encountered when these were dissolved in phosphate buffer instead of twice distilled water.

CPB : SE(S) mice (TNO, Zeist, The Netherlands) of either sex 20–25 g were maintained at 21–23°. Coded test solutions were administered intraperitoneally in a dose volume of 0·1 ml per mouse. After the challenge each mouse was separately placed in a translucent cage kept in a quiet environment and the number of constriction responses in the time 10–20 min after the injection counted, as this interval gave the most reproducible results after a challenge. In 4 experiments with different dilutions of acetic acid, in twice distilled water, and using the 10–20 min observation period a fair dose response relation was found (Fig. 1) showing the test model gave a quantitative and reproducible result.

We then used lipopolysaccharides (LPS) from some Gram-negative bacteria. In experiments with the LPS prepared from *Escherichia coli*, *Salmonella typhosa*, *S. typhimurium* or *S. enteritidis* (Difco Laboratories, Detroit, Michigan) it soon became apparent that these polymers in twice distilled water were as slow-acting as acetic acid and elicited only a low frequency of responses. As preparations for parenteral use are sometimes buffered, we then dissolved the LPS in phosphate buffer 0·12 M, pH 7·2 and observed much stronger responses. The results of 9 independent experiments with the four LPS each dissolved in twice distilled water or phosphate buffer at widely different concentrations are given in Fig. 2. It is clear that the use of phosphate buffer has a profound enhancing effect on the frequency of the constriction responses. This effect of phosphate is evident over a dose range extending from 5×10^{-1} to 5×10^{4} ng of LPS per mouse and it is quantitatively the same with all four LPS substances.

In a separate experiment in which 24 mice per group were used, the dose of LPS from *E. coli* was kept constant at 5×10^{2} ng per mouse but dissolved in

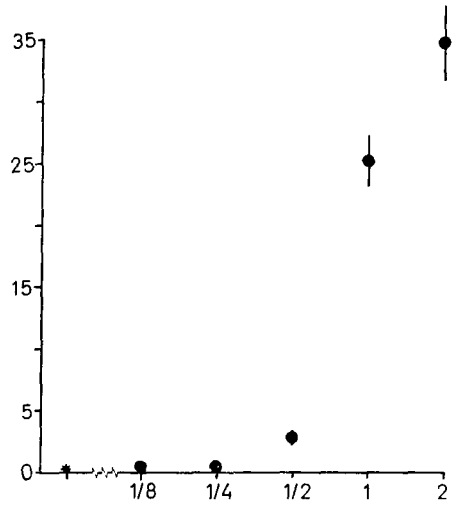

FIG. 1. Dose response relation with acetic acid diluted with twice distilled water. Composite data from 4 experiments. One expt with all 5 doses of acetic acid, the other 3 expts with only the 3 highest doses. In all expts the groups consisted of 24 mice. The symbols are means with ±s.e.m. when appropriate (at the low scores the individual responses per group do not demonstrate a normal distribution because of the presence of zero values (non-responding mice). In such cases it is either not possible to calculate the s.e.m. or they are only 'approximate' values). The mean of the control groups (4 × 24 mice) with saline treatment is also shown. Ordinate: Abdominal constriction response. Abscissa: Acetic acid (% w/v). The star represents the saline control.

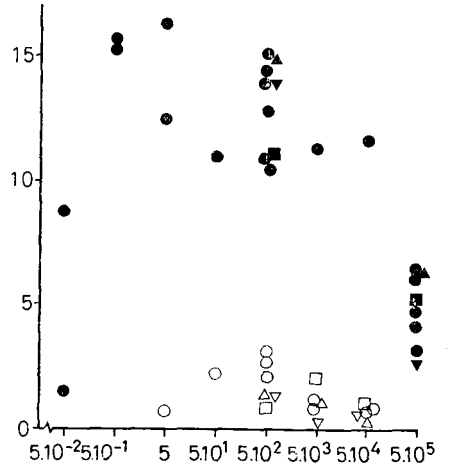

FIG. 2. Phosphate effect on the abdominal constriction response to the intraperitoneal injection of LPS. The LPS were dissolved in phosphate buffer (black symbols) or in twice distilled water (open symbols). Each symbol represents the mean score of 12 mice. The different symbols refer to LPS from *E. coli* (●, ○), *S. typhosa* (▲, △), *S. typhimurium* (▼, ▽) and *S. enteritidis* (■, □). Composition of the phosphate buffer: 4·12 g KH_2PO_4 and 15·45 g $Na_2HPO_4 . 2H_2O$ litre^{-1} of twice distilled water. The 2 solvents were tested and showed negligible constriction response scores. Ordinate: Abdominal constriction response. Abscissa: ng LPS per mouse.

different mixtures of water and phosphate buffer. The results (Fig. 3) clearly confirm the enhancing effect of phosphate on the response and further demonstrate that it is related to the concentration of phosphate.

The pH values of the test preparations were different. The pH of the twice distilled water ranged from 5·1 (blank medium) to 5·4 at the highest LPS concentration tested, whereas the phosphate buffer used had a pH of 7·2. This difference might account for the observed effect of phosphate. However, control tests with LPS dissolved in lightly phosphate buffered (6 mM) saline (pH 7·2) demonstrated that the responses to LPS were similar to those obtained when LPS were dissolved in water. It is therefore evident that the difference between the pH values of the solutions is not responsible for the effect of phosphate on the response to LPS. The solvents themselves do not produce constriction responses or only occasional ones in some mice.

At doses above 5 × 10^4 ng LPS per mouse the constriction scores decrease (Fig. 2). At these high doses the mice also change their behaviour, the typical abdominal constriction responses fade, the animals withdraw and show lowered mobility indicating that some other factor depresses the constriction response.

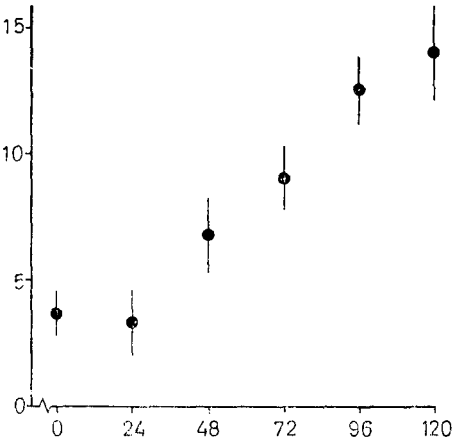

FIG. 3. Effect of phosphate concentration on the abdominal constriction response. In all cases the *E. coli*-LPS dose was 5 × 10^2 ng per mouse. The solvent mixtures were water, with increasing quanta of phosphate buffer (0·12 M). Each symbol is the mean constriction score of 24 mice ± (approximate) s.e.m. The 2 solvents showed negligible constriction response scores. Ordinate: Abdominal constriction response. Abscissa: Phosphate concn (mM).

The enhancing effect of phosphate on the constriction response is not specific for LPS. It could be demonstrated also with phenyl-*p*-quinone at doses of 20 and 40 μg per mouse.

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Interaction between doxapram and pentobarbitone in the mouse

BARBARA J. PLEUVRY*, STEPHANIE E. MADDISON, B-B. SAHAL, *Departments of Anaesthetics and Pharmacology, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.*

The classification of doxapram as a non-specific analeptic (e.g. Goodman & Gilman, 1975) has been challenged by Cohn (1974) and Kraynack, Cohn & others (1976), who found that doxapram potentiated amylobarbitone sleeping time in the rat. This result was in contrast to those for other 'analeptics' such as nikethamide, which had no effect on amylobarbitone sleeping time and picrotoxin which reduced amylobarbitone sleeping time.

The present study was designed to determine whether there was an interaction between the barbiturates and doxapram in mice and if so to examine some possible mechanisms for this interaction.

Female mice (25–30 g) of the Manchester Strain were used and all drugs were administered intraperitoneally.

After injection of pentobarbitone sodium, 30 mg kg^{-1} the time to onset of ataxia and loss of righting reflex (induction time) was noted. Sleeping time was measured as the duration of loss of righting reflex. Finally the time to full recovery, i.e. no discernible ataxia or sedation, was noted. Since this last parameter was extremely subjective a second observer with no knowledge of which drugs had been injected assessed the time to full recovery.

In separate experiments, respiratory rates were measured using the method of Bousfield & Rees (1969). Blood barbiturate concentrations were measured using the method of Brodie, Burns & others (1953). Each determination was made on the pooled blood from 4 mice killed 30 min after the pentobarbitone injection. LD50 values for doxapram were calculated using the method of Litchfield & Wilcoxon (1949). 20 mice were used for each dose of drug and observations were continued for 24 h.

Mice receiving both pentobarbitone sodium and doxapram hydrochloride were given both drugs simultaneously. In control experiments when SKF 525A was used, this drug was injected 45 min before the barbiturate.

Doxapram caused a dose-dependent increase in the hypnotic action of pentobarbitone (Table 1). The lowest dose used, 10 mg kg^{-1} doxapram caused a significant prolongation of the time necessary for full recovery, whilst the highest dose used, 100 mg kg^{-1}, decreased induction time, increased sleeping time and prolonged the time to full recovery. Mice given these doses of doxapram alone did not become noticably ataxic, nor did they loose their righting reflex.

Potentiation of the actions of pentobarbitone by doxapram was also seen in the respiratory rate measurements. Table 2 shows the change in respiratory rate in mice treated with 100 mg kg^{-1} doxapram and 30 mg kg^{-1} pentobarbitone 30 min after injection. Doxapram also

Table 1. *Effects of doxapram and SKF 525A on the hypnotic activity of an intraperitoneal injection of 30 mg kg^{-1} pentobarbitone in the mouse.*

	Time (min): mean (range) n = 18		
	Induction†	Sleep	Full recovery
Doxapram 100 mg kg^{-1}	6·9 (3·3–∞)**	38·8 (0–140)**	All > 240**
Concurrent controls	8·8 (6·6–∞)	10·0 (0–20·4)	59 (47–70)
Doxapram 50 mg kg^{-1}	7·1 (5·0–∞)	26·9 (0–90)*	(180–>240)**
Concurrent controls	7·9 (4·7–13·2)	16·8 (2·9–42)	62·5 (45–85)
Doxapram 25 mg kg^{-1}	9·7 (7·0–14·0)	16·3 (9·1–32·0)	150·5 (90–205)**
Concurrent controls	10·4 (6·0–15·1)	15·0 (7·0–23·9)	76·9 (60–93)
Doxapram 10 mg kg^{-1}	10·6 (7·1–14·9)	7·9 (4·0–17·3)	63·9 (53–75)**
Concurrent controls	9·8 (8·0–13·0)	7·1 (4·1–15·0)	52·9 (41–75)
SKF 525A 10 mg kg^{-1}	7·2 (4·9–10·5)**	86·8 (55–144)**	All > 240**
Concurrent controls	10·6 (6·0–∞)	13·5 (0–43·6)	79·0 (63–91)

* $0·1 < P > 0·5$; ** $P < 0·05$ compared with concurrent control values using the Mann Whitney 'U' test.
† Means exclude mice not losing their righting reflex.

* Correspondence.