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RESTORATION OF MEAN ARTERIAL PRESSURE IN ENDOTOXIC SHOCK BY MEPTAZINOL

CONTRIBUTIONS FROM LYSOSOMAL MEMBRANE STABILISATION, OPIATE ANTAGONISM AND NORADRENALINE RELEASE

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Abstract—Meptazinol elevated mean arterial pressure in rats which had been treated with endotoxin. The drug also reduced the titer of circulating lysosomal enzymes. This effect was secondary to the restoration of mean arterial pressure (MAP). *In vitro*, meptazinol stabilised lysosomal membranes, increased noradrenaline release and interacted with the opiate receptor (naloxone-binding site) as an antagonist. The relevant contributions of these phenomena to the restoration of MAP are discussed.

Lysosomal enzymes are released into the systemic circulation following various types of trauma including haemorrhagic [1], burn [2] or endotoxic shock [3]. The significance of the release and action of these enzymes to the onset and duration of shock is unclear, but agents which inhibit lysosomal enzyme release, i.e. methylprednisolone, indomethacin and chlorpromazine, produce beneficial cardiovascular responses [4, 5]. The opiate antagonist naloxone also produces a pressor response in shock, both in experimental animals [6] and in man [7]. This effect may be due to either central or peripheral antagonism of cardiodepressant opiates [8] with resultant alterations in autonomic efferent activity [9]. In addition, naloxone stabilises lysosomal membranes in shock [10]; however, this action may be a consequence of increased perfusion of vital organs since alpha adrenoceptor antagonists such as phenoxybenzamine protect animals against shock without possessing membrane-stabilising properties [11].

Meptazinol, a clinically effective opioid antagonist analgesic [12], increases blood pressure in endotoxic shock [13] and is compared here with pentazocine and a number of agents known to stabilise lysosomal membranes. These experiments were designed to determine whether the blood pressure effects correlate with acid phosphatase release *in vivo* and *in vitro*. In addition, agents which increased blood pressure were examined for their ability to increase noradrenaline release from isolated nerve terminals (synaptosomes).

METHODS

Female Sprague–Dawley rats (Charles River) weighing between 200 and 250 g were anaesthetised with a mixture of urethane (800 mg/kg) and chloralose (60 mg/kg) in a vol. of 10 ml/kg intraperitoneally. Blood pressure was recorded using a Statham P23D transducer from a cannula inserted into the left carotid artery and displayed on a Grass polygraph. Heart rate was derived from the blood pressure signal by a tachograph. All values are for mean arterial pressure (MAP).

The left jugular vein was cannulated for drug administration and animals were allowed to breathe spontaneously via a tracheal cannula. Deep body temp was maintained at 37 ± 0.5° using a heating blanket. The rats were allowed to stabilise for 30 min before administration of *E. coli* endotoxin (Difco, serotype 055:B5) intravenously (i.v.) by infusion over 20 sec. Drugs or saline were administered 90 min later and blood pressure and heart rate measured for a further 30 min. Blood samples (5-ml) were removed at the end of the experiment for determination of acid phosphatase activity. In an initial series of experiments acid phosphatase activity and the haematocrit were measured at 15-min intervals in control animals (N = 4) and in animals which had received endotoxin (10 mg/kg i.v.) (N = 4). Groups of four animals each received chlorpromazine (0.025, 0.25 and 2.5 mg/kg); methylprednisolone (25 mg/kg), indomethacin (2 mg/kg) or naloxone (10 mg/kg) i.v. Meptazinol (2,6 and 17 mg/kg) and pentazocine (3, 10 and 30 mg/kg) were administered intramuscularly. Blood pressures and heart rates were compared before and after drug administration with a control group which received 0.9% saline (1 ml/kg) i.v. The activity of the agents administered *in vivo* was also studied *in vitro* using a lysosomal fraction isolated by differential centrifugation from the livers of rats (Sprague–Dawley) weighing between 150 and 200 g [14]. The lysosomes were resuspended in Krebs bicarbonate medium at pH 7.4 and incubated at 37° for 60 min. The suspensions were then centrifuged at 10,000 *g* for 30 min and aliquots of supernatant assayed for acid phosphatase activity. A range of drug concns was added to the incubation medium and appropriate control incubations were carried out to determine the total amount of enzyme released by 0.1% v/v Triton X-100.

Plasma and liver lysosomal enzyme activities were

assayed as described in the Sigma technical bulletin No. 104, based on the release of *p*-nitrophenol from the substrate *p*-nitrophenol phosphate. Samples were either assayed immediately (liver) or stabilised by the addition of citric acid and stored at 4° prior to assay (plasma). Acid phosphatase activity was expressed as Sigma units/ml.

Synaptosomes were prepared by differential and density gradient centrifugation [15] and suspended in a medium containing (mM): NaCl, 136; KCl, 5; $MgCl_2$, 2.5; $CaCl_2$, 2.5; glucose, 10; ascorbate, 1; nialimide, 0.02; and Tris, 20. The pH was adjusted to 7.4 with HCl and the medium gassed with 100% O_2 for 30 min before use. The release of noradrenaline measured fluorimetrically is described in detail elsewhere [15]. Protein concn was determined by the method of Lowry *et al.* [16].

Values for blood pressure were compared by nested analysis of variance, *in vivo* plasma acid and *in vitro* liver lysosomal acid phosphatase by unpaired *t*-test.

Drugs. Chlorpromazine hydrochloride (May & Baker), methylprednisolone (UpJohn), meptazinol hydrochloride (Wyeth), naloxone hydrochloride (Endo) and pentazocine lactate (Sterling Winthrop) were administered in 0.9% saline. Indomethacin (Sigma) was dissolved in a small volume of absolute ethanol and made up in 0.9% saline with phosphate buffer (pH 8.0).

RESULTS

Endotoxin administration produced an immediate decrease in MAP which was maximal at 30 min (Fig. 1) and also correlated with a maximal increase in the haematocrit. At this time there was also a significant increase in plasma acid phosphatase activity which however did not reach a maximum during the course of the experiment. The haematocrit and acid phosphatase activity increased by 21% 30 min following endotoxin administration, whereas 120 min after endotoxin there was no further increase in the haematocrit but acid phosphatase activity was increased by 42% (Fig. 1). These results indicate that the increased acid phosphatase activity is probably not a consequence of decreased plasma volune *per se* and is secondary to a drop in blood pressure.

Administration of saline vehicle (i.v.) produced a small increase in blood pressure presumably due to some spontaneous recovery of blood pressure between 90 and 120 min.

Chlorpromazine at 0.025 and 0.25 mg/kg did not affect plasma acid phosphatase activity or blood pressure whereas at 2.5 mg/kg it decreased blood pressure without affecting plasma acid phosphatase activity (Table 1).

Meptazinol at 2 mg/kg produced a significant ($P < 0.001$) reduction in plasma acid phosphatase activity without affecting blood pressure whereas higher doses reduced enzyme release (Table 1) and increased blood pressure. Although there was a dose-related increase in blood pressure after meptazinol. Higher doses did not significantly reduce acid phosphatase activity below that produced by 2 mg/kg.

Pentazocine at 3 and 30 mg/kg produced no effect on blood pressure whereas at 10 mg/kg it produced a small but significant reduction. Pentazocine at 3 mg/kg produced a small but significant reduction in plasma acid phosphatase activity (Table 1).

Indomethacin increased blood pressure significantly ($P < 0.05$) by 19 mmHg without affecting plasma acid phosphatase activity (Table 1). This increase in blood pressure was similar to that produced by meptazinol (6 mg/kg). Methylprednisolone produced a significant ($P < 0.001$) increase in blood pressure of 13.5 ± 2.7 mmHg and produced a marked decrease in acid phosphatase activity (Table 1).

The release of acid phosphatase from liver homogenates incubated under physiological conditions represented 10.3 ± 0.4% of the enzyme released by Triton X-100. The total releasable acid phosphatase was not influenced by any of the drugs used. The release of enzyme in the absence of Triton X-100 was however subject to drug modification (Fig. 2). Chlorpromazine stabilised lysosomes at low concns but increased acid phosphatase release at concns above 10^{-4} M. Meptazinol and methylprednisolone produced membrane stabilisation at all concns tested and naloxone produced similar effects at concns above 10^{-7} M (Fig. 2). The order of potency of the agents studied as membrane stabilisers was methylprednisolone < chlorpromazine < naloxone < meptazinol. Indomethacin and pentazocine had no significant effect on lysosomal membrane stability.

Synaptosomal noradrenaline release was stimu-

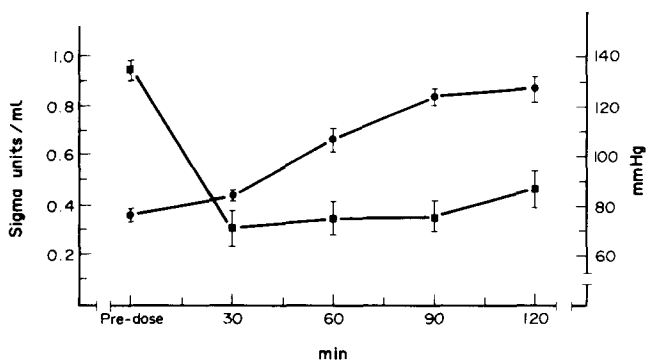

Fig. 1. The effect of endotoxin (10 mg/kg) on plasma acid phosphatase activity (●) and blood pressure (■) in anaesthetised rats. All values are means ± S.E.M. (N = 4).

Table 1. Effect of meptazinol and standard agents on mean arterial pressure (MAP) and plasma acid phosphatase activity following endotoxin administration (10 mg/kg) to anaesthetised rats

Drug	Dose (mg/kg)	MAP (mmHg)			Acid phosphatase activity (Sigma units/ml)
		Time relative to endotoxin (min)			
		0 (Pre-endotoxin)	90	120	120 min
Vehicle		129 ± 4.7	79.6 ± 5.1	87.1 ± 2.4	0.90 ± 0.02
Meptazinol	2.0	117 ± 8.6	79.6 ± 3.4	92.1 ± 1.4	0.67 ± 0.02***
Meptazinol	6.0	129 ± 5.6	82.1 ± 6.3	102.0 ± 7.9***	0.53 ± 0.04***
Meptazinol	17.0	123 ± 6.2	75.4 ± 4.0	101.0 ± 3.4***	0.77 ± 0.02**
Chlorpromazine	0.025	121 ± 6.9	78.4 ± 2.8	86.7 ± 3.0	0.87 ± 0.07
Chlorpromazine	0.25	129 ± 6.6	85.0 ± 4.8	80.0 ± 4.1*	0.90 ± 0.03
Chlorpromazine	2.5	132 ± 7.6	83.8 ± 6.5	64.6 ± 7.5***	0.86 ± 0.02
Pentazocine	3.0	123 ± 0.7	78.8 ± 6.5	88.4 ± 5.6	0.81 ± 0.02
Pentazocine	10.0	137 ± 5.1	77.1 ± 4.5	80.0 ± 7.1*	0.82 ± 0.03
Pentazocine	30.0	121 ± 8.0	77.9 ± 5.9	89.2 ± 4.0	0.84 ± 0.02
Naloxone	10.0	127 ± 6.2	81.7 ± 3.0	104.0 ± 4.7***	0.67 ± 0.01***
Methylprednisolone	25.0	116 ± 10	89.2 ± 0.9**	103.0 ± 3.5***	0.64 ± 0.04***
Indomethacin	2.0	125 ± 4.5	77.1 ± 7.5	95.8 ± 12*	0.91 ± 0.04

All drugs were administered 90 min after endotoxin.
The time scale refers to events subsequent to endotoxin administration.
All values are the means ± S.E.M. of four to eight experiments.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

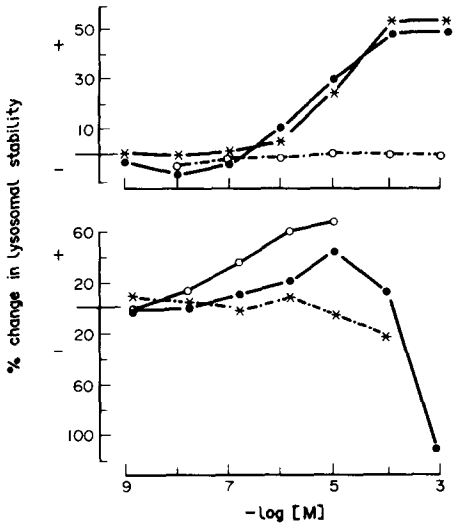

Fig. 2. Alterations in lysosomal membrane stability by various drugs. All results are the means of four to six determinations. S.E.s, calculated from the absolute values, were all less than 7% of the mean. Lysosomal stabilisation is denoted as (+), whereas lysis is denoted by (−). Upper graph: (✱)—meptazinol, (●)—naloxone, (○)—pentazocine. Lower graph: (✱)—indomethacin, (○)—methylprednisolone, (●)—chlorpromazine.

lated by up to 80% under conditions of K^+ depolarisation (Table 2). The basal and stimulated release of noradrenaline were increased in a concn-dependent manner by meptazinol and indomethacin. Maximal increment was observed at 10^{-8} M meptazinol and 10^{-6} M indomethacin (Table 2). Basal noradrenaline release was increased by naloxone whereas methylprednisolone increased only stimulated release. Chlorpromazine inhibited noradrenaline release at concns above 10^{-5} M (Table 2). Pentazocine inhibited basal and stimulated release of noradrenaline in the concn range 10^{-8}–10^{-6} M. The effects were maximal at 10^{-7} M.

DISCUSSION

The mechanisms involved in the pressor effect of naloxone in shock are currently under debate and possibilities include antagonism of endogenous opiates [6], stabilisation of lysosomal membranes [10] or release of catecholamines [17]. Meptazinol, like naloxone, indomethacin and methylprednisolone produced a pressor effect in animals following endotoxic shock. In marked contrast, relatively high doses of chlorpromazine and pentazocine reduced MAP relative to vehicle. With the exception of pentazocine and indomethacin all these drugs produced reductions in circulating plasma acid phosphatase. This was maximal with meptazinol at a dose of 6 mg/kg; a lower dose produced an equivalent reduction in acid phosphatase to that observed with naloxone or methylprednisoline.

Similar results were obtained *in vitro* so that naloxone, meptazinol, methylprednisolone and chlorpromazine produced concn-dependent stabilisation of lysosomal membranes. These results confirm those of previous workers [5, 18], and indicate for the first time that meptazinol has lysosomal membrane stabilising properties. This activity, which is shared by naloxone, appears not to be a feature of opiate analgesics of the partial agonist type, since pentazocine neither increased blood pressure nor reduced acid phosphatase release *in vivo* or *in vitro*.

Changes in acid phosphatase occurred after blood pressure had decreased in this endotoxic shock model and reached a plateau 120 min after endotoxin administration. The ability of several of the agents to decrease plasma levels when administered after endotoxin administration indicates that release is occurring throughout the experiment. The activities of two of the drugs examined, indomethacin and chlorpromazine, indicate that there is probably more than just a temporal discrepancy between alterations in blood pressure and lysosomal membrane stabilisation.

Indomethacin increased blood pressure without affecting acid phosphatase and chlorpromazine reduced blood pressure at doses which did not affect acid phosphatase release *in vivo*. Changes in acid phosphatase are probably secondary to shock [19] and increased acid phosphatase could therefore be a consequence of inadequate perfusion. The discrepancy in the direction of blood pressure change with chlorpromazine may indicate that this drug pro-

Table 2. The influence of drugs on noradrenaline release from rat cortical synaptosomes

Drug	Concn† (μM)	Noradrenaline release (nmoles/g protein)	
		Basal	Stimulated
Control	—	32 ± 2.7	58 ± 2.8
Meptazinol	0.01	39 ± 1.7**	73 ± 1.4***
Naloxone	0.01	38 ± 1.8*	61 ± 1.4
Methylprednisolone	0.01	34 ± 1.8	68 ± 1.6***
Indomethacin	1.0	36 ± 0.7	67 ± 2.0**
Pentazocine	0.1	26 ± 2.0*	47 ± 3.4*
Chlorpromazine	100.0	24 ± 2.6*	45 ± 1.9**

† Concns are the lowest required to produce the maximum effect.
All results are the means ± S.E.M. of four experiments except for control (N = 14).
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The level of significance was determined by unpaired *t*-test relative to the control for that experiment only.

duces vasodilation [20]. The use of blood pressure *per se* does not however indicate whether perfusion itself is altered. While lysosomal membrane stabilisation is an attractive working hypothesis to describe the beneficial action of a diverse series of compounds, alternative sites must be considered.

Naloxone interacts with opiate receptors as an antagonist [21]. Similar experiments with receptor binding reveal that meptazinol displays the characteristics of an antagonist with a sodium ratio of 1.4–1.7 [22, 23]. Pentazocine has a ratio of 6, characteristic of a partial agonist. There is no evidence however of a significant interaction between the opiate receptor and either indomethacin or methylprednisolone; therefore an interaction with opiate receptors cannot be satisfactorily involved as a unitary hypothesis. It is pertinent to note that all agents which raised blood pressure *in vivo* increased noradrenaline release from rat cortical synaptosomes *in vitro*. The increment in neurotransmitter release could be due to inhibition of endogenous enkephalins which decrease noradrenaline release (naloxone and meptazinol), blockade prostaglandin release and/or synthesis which inhibits noradrenaline release (indomethacin) or by blockading uptake (methylprednisolone) [24–26]. Increased circulating catecholamines maintain arterial blood pressure in shock [8] and a further increased release may induce a pressor response. Antagonism of catecholamines by chlorpromazine could therefore be involved and would explain the reduction in MAP observed. The significance of increased peripheral noradrenaline release in shock is however equivocal.

As part of normal autonomic compensatory mechanisms to a reduction in MAP, noradrenaline release would presumably be already at maximum. Naloxone is effective in shock when administered intracerebroventricularly [3] and alterations in central noradrenergic function may be of more relevance.

While further experiments are required to resolve mechanisms of action, it is clear that meptazinol, a clinically effective opioid antagonist analgesic [12], compares favourably with methylprednisolone and naloxone in the treatment of endotoxic shock.

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