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Antagonism of the Cardiovascular and Respiratory Depressant Effects of Morphine in the Conscious Rabbit by Physostigmine[1]

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ABSTRACT

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The influence of physostigmine was studied on the effect of morphine on the cardiovascular and respiratory systems in conscious rabbits. Morphine (4 mg/kg i.v.) caused analgesia, bradycardia, hypotension and respiratory depression, as indicated by a fall in respiratory rate of 50%, a rise in blood Pa_{CO_2} from 25.1 to 37.2 mm Hg and a fall in pH from 7.40 to 7.24. These effects lasted 2 to 3 hr and were completely antagonized by naloxone. Physostigmine (2.5 or 5 μg/kg/min) given by constant i.v. infusion did not significantly alter blood pressure or heart rate, but decreased blood Pa_{CO_2} from 25.1 to 19 mm Hg and increased pH from 7.40 to 7.46. Pretreatment of rabbits with physostigmine (5 μg/kg/min) completely prevented both the fall in blood pressure and blood pH and the rise in Pa_{CO_2} induced by morphine (4 mg/kg) and also significantly reduced both the intensity and duration of bradycardia. Analgesic activity of morphine remained unimpaired by physostigmine. Neostigmine (2.5 μg/kg/min) potentiated the bradycardia induced by morphine and did not antagonize its hypotensive and respiratory depressant effects. The results support the hypothesis that the respiratory and cardiovascular depressant effects of morphine, but not the analgesia, result from an inhibition of acetylcholine release from neurons in the central nervous system.

Injection of acetylcholine and other muscarinic agonists into the lateral ventricle or cisterna magna of dogs and rats results in a rise in blood pressure and tachycardia (Lang and Rush, 1973; Sinha *et al.*, 1967; Krstic and Djurkovic, 1978). Hypertension also results from direct electrical stimulation of the vasomotor areas in the medulla, and this can be prevented by hemicholinium (Sinha *et al.*, 1967). Application of acetylcholine to the floor of the 4th ventricle markedly increased respiration rate in decerebrate cats, pretreated with physostigmine (Miller, 1949). These data suggest the presence of cholinergic stimulatory pathways to the vasomotor and respiratory centers.

Morphine and related opiates can increase acetylcholine levels in the brain. This does not result from stimulation of acetylcholine synthesis or from prevention of acetylcholine hydrolysis, but is most likely due to an inhibition of acetylcholine release (Weinstock, 1971). Such an action has been demonstrated for several opiate drugs in different areas of the central nervous system (Beleslin and Polak, 1965; Jhamandas *et al.*, 1971; Domino and Wilson, 1973; Zsilla *et al.*, 1976). Furthermore, morphine is known to produce effects such as respiratory depression, hypotension and bradycardia, which are the opposite to those caused by central cholinergic receptor stimulation. It therefore seemed reasonable to suggest that these effects of morphine result from impairment of acetylcholine release.

Previous studies which have attempted to show a correlation between the cardiovascular or respiratory depressant effects of morphine and acetylcholine release, have been carried out in anesthetized animals (Schaumann, 1958; Laubie *et al.*, 1974). Anesthetic agents, particularly barbiturates, markedly interfere with central cholinergic activity as well as with respiratory control (Bradley and Dray, 1973; Borison, 1971; Weinstock *et al.*, 1979).

The present study was therefore carried out in conscious animals. These were pretreated with physostigmine, which increases the amount of acetylcholine available for interaction with its receptors by blocking acetylcholinesterase. If such pretreatment were found to antagonize the cardiovascular and respiratory depressant actions of morphine, it would lend support to the suggestion that interference with cholinergic transmission was involved in these effects of morphine.

Methods

Measurement of cardiovascular and respiratory parameters. Male rabbits (mixed strain) weighing 2.5 to 3.5 kg were trained to sit quietly in a restraining box. Both ear arteries were cannulated with transcutaneous catheters (Quick Cath. No. 20, Travenol Laboratories Ltd., Castlebar, Ireland) which were filled with sterile saline containing

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25 μ/ml of heparin. Blood pressure and heart rate were recorded on a Brush Gould recorder by means of a transducer attached to one arterial cannula. Drugs were administered through a butterfly needle (no. 23) placed in a marginal ear vein. Physostigmine or neostigmine was infused i.v. in a volume of 0.09 ml/min by means of a Harvard constant infusion pump.

Rectal temperature was monitored on a telethermometer (Yellow Springs Instrument Company, Yellow Springs, OH) with the aid of a thermistor probe inserted into the rectum. Respiration rate was counted visually and blood gases and pH were measured on Corning automatic blood gas analyzer after adjustment to the appropriate body temperature.

Analgesia was assessed from the reaction (squeal or attempt at withdrawal) in response to one of four grades of pressure applied to the tip of the tail with a sponge holder clamp. A score of 1 indicated a positive reaction to the lowest degree of pressure, whereas 5 denoted that no reaction to the highest degree of pressure occurred.

Rabbits were allowed to rest for at least 1 hr under quiet conditions after cannulation, before control readings were taken. Drugs were not administered until two consistent values for blood gases were obtained.

Mean arterial blood pressure, heart and respiration rates were determined at 10-min intervals for 1 hr and then at 30-min intervals for 2 hr after injection of morphine. Six rabbits were pretreated with ATMN (0.5 mg/kg i.v.) and then were given morphine (4 mg/kg) as above. In other rabbits, physostigmine was infused at a concentration of either 2.5 (seven animals) or 5 μg/kg/min (eight animals). Thirty minutes after commencement of the infusion, morphine was injected slowly over a period of 2 to 3 min and the infusion of physostigmine continued for an additional 2 hrs. Blood samples (0.6 ml) for blood gas analysis were taken at least twice before drug administration, 30 min after infusion of physostigmine, and at 30, 60 and 90 min after injection of morphine. The volume of blood taken was replaced each time with an equal volume of sterile saline. In five rabbits, neostigmine (2.5 μg/kg/min) was infused for 30 min and then continued after injection of morphine (4 mg/kg). Blood pressure, heart and respiration rates and blood gases were measured as above. In six other rabbits, ATMN (0.5 mg/kg), or in four animals, hyoscine (10 mg/kg), was given, 15 min before the infusion of physostigmine, 5 μg/kg/min.

In four rabbits, naloxone was infused i.v. at a concentration of 0.1 mg/kg/min for 15 min before and for 90 min after injection of 4 mg/kg of morphine. Blood pressure, heart and respiration rates were recorded as described above.

Estimation of plasma cholinesterase. Blood (0.3–0.5 ml) was withdrawn into a heparinized syringe during the predrug control period and at 30 and 60 min after commencement of physostigmine infusion (*i.e.*, 30 min after morphine injection). The blood was centrifuged immediately for 10 min at 1000 × *g* and cholinesterase activity of the plasma was measured within 10 min by the method of Ellman *et al.* (1961). Drugs used were: ATMN, hyoscine hydrobromide, neostigmine hydrobromide and physostigmine salicylate (Sigma Chemical Company, St. Louis, MO); morphine hydrochloride (U.S. Vitamins Laboratories Division, Tuckahoe, NY); and naloxone hydrochloride (Endo Laboratories, Inc., Garden City, NY). Morphine and physostigmine were made up freshly for each experiment in sterile saline which included an equal weight of ascorbic acid to prevent oxidation. All doses are expressed in milligrams per kilogram of body weight of the appropriate salt.

Statistical analysis. Tests of significance for the difference between means were performed by a two-tailed Student's *t* test for paired or unpaired data as indicated in "Results".

Results

Effect of morphine on blood pressure, heart rate, respiration and pain threshold. Intravenous injection of morphine (2 mg/kg) caused significant bradycardia (reduction of 72 ± 10 beats/min) within 5 min, whereas 1 mg/kg only reduced heart rate by 30 ± 9 beats at 60 min. Reductions in heart rate

injection of 4 and 10 mg/kg of morphine respectively. The peak hypotensive response (9.8 ± 2.2 and 12.4 ± 1.8 mm Hg) occurred 20 to 30 min after injection of 2 and 4 mg/kg, respectively. The response to 10 mg/kg of morphine was inconsistent, with some rabbits displaying a rise of 5 to 10 mm Hg during the first 10 min and others, a small nonsignificant fall. Both the bradycardia and hypotensive response to 4 mg/kg of morphine lasted 2.5 to 3 hr.

A dose of 4 mg/kg of morphine was therefore chosen for all subsequent experiments since it produced the most extensive and consistent vasodepression and bradycardia.

Pretreatment with ATMN (0.5 mg/kg) completely prevented the bradycardia and reduced the fall in blood pressure.

Morphine (4 mg/kg) caused more than a 50% reduction in respiratory rate, which was associated with a 48% increase in arterial Pa_{CO_2}. Blood pH was reduced from 7.40 to 7.24 (see fig. 1). Maximum respiratory depression occurred between 30 and 60 min after morphine administration and lasted 3 hr.

A considerable degree of analgesic activity was also seen at this dose level, 30 min after injection of morphine, with most of the rabbits failing to respond to the highest degree of pressure (table 1).

Naloxone, given by continuous i.v. infusion at a dose of 0.1 mg/min completely prevented all the above effects of morphine (4 mg/kg) in four rabbits.

Influence of anticholinesterase agents on actions of

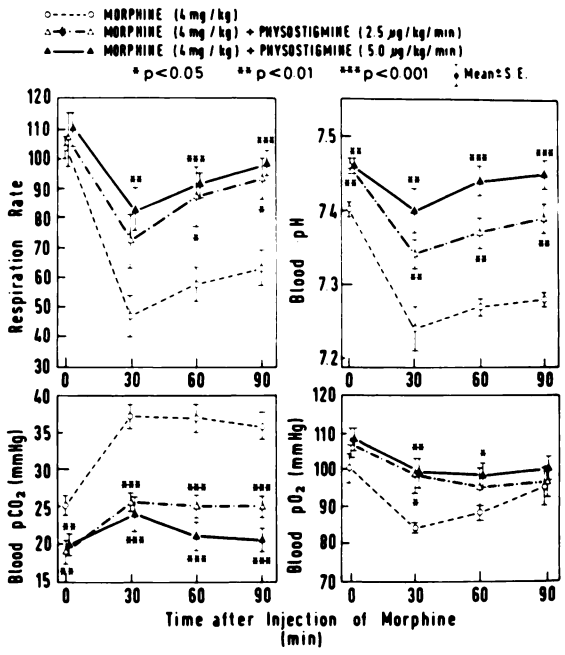

Fig. 1. The effect of physostigmine pretreatment on the respiratory depression induced by morphine.

TABLE 1

The effect of physostigmine on analgesic activity of morphine

Treatment	*N*	Analgesic Score ± S.E.M.
Control	21	1.38 ± 0.13
Morphine (4 mg/kg)	9	4.55 ± 0.24
Physostigmine (5 μg/kg/min, 30 min)	7	1.71 ± 0.28
Physostigmine (5 μg/kg/min, 30 min) + morphine	12	4.91 ± 0.08

morphine. Intravenous injection of physostigmine in doses of 0.05 to 0.2 mg/kg resulted in biphasic effects on blood pressure and heart rate, which lasted 20 to 30 min, depending on the dose. An initial phase of bradycardia and hypotension was followed by a longer phase of hypertension and return of heart rate to normal values. Since one could not study the cardiovascular effects of morphine in the presence of continuously changing and short-lived effects of physostigmine, it was decided to give physostigmine as a continuous i.v. infusion. When administered in this way at a concentration of 2.5 or 5 μg/kg/min, physostigmine caused no significant change in either heart rate or blood pressure over a period of 2 hr (table 2). Both doses of physostigmine caused miosis and some salivation and defecation 20 to 40 min after commencement of the infusion. The effects occurred earlier and were more pronounced with the higher dose.

Both concentrations markedly stimulated respiration, resulting in an increase in blood pH and a 20 to 25% decrease in Pa_{CO_2} within 30 min of commencement of the infusion (table 2; fig. 1). Plasma cholinesterase was inhibited by 48.4 and 55.3% at 30 min by physostigmine, 2.5 and 5 μg/kg/min, respectively. There was no significant additional inhibition of plasma cholinesterase at either dose of physostigmine at 60 min. Slight muscle fasciculations were only evident 2 hr after continuous infusion of the larger dose. In four other rabbits given physostigmine (10 μg/kg/min), considerable salivation and defecation occurred within 30 min and muscle fasciculations appeared 40 to 60 min after commencement of the infusion. This dose was therefore not given together with morphine.

When physostigmine infusion (2.5 or 5 μg/kg/min) was given for 30 min and then continued after the injection of morphine (4 mg/kg), it completely prevented the fall in blood pressure. The bradycardia was not significantly altered by the lower dose of physostigmine, but it was reduced both in intensity and duration after administration of 5 μg/kg/min of the anticholinesterase (see fig. 2).

Pretreatment with physostigmine (2.5 μg/kg/min) markedly diminished the elevation in Pa_{CO_2} which occurred after administration of morphine (4 mg/kg). It also reduced significantly the fall in blood pH, respiration rate and Pa_{O_2} 60 min after morphine (see fig. 1). After pretreatment with physostigmine (5 μg/kg/min), morphine no longer caused any significant change in blood Pa_{CO_2}, pH or Pa_{O_2} and the fall in respiratory rate was greatly diminished (see table 3).

Pretreatment of rabbits with ATMN (0.5 mg/kg) did not significantly alter the respiratory depressant effect of morphine given alone, nor did it alter the prevention of respiratory depression by physostigmine (5 μg/kg/min) (see table 3). It only antagonized the salivation, miosis and defecation induced by physostigmine. On the other hand, hyoscine, 10 mg/kg, both increased the respiratory depressant effect of morphine alone (P_{CO_2} at 30 min after morphine, 40 ± 1.2) and completely antagonized the prevention by physostigmine of the respiratory depressant effect of morphine (see table 3).

Neostigmine (2.5 μg/kg/min) inhibited plasma cholinesterase by 80%, but it did not diminish the respiratory depressant effect of morphine, nor did it alter significantly the hypotensive response. However, it markedly potentiated the cardiac slowing induced by morphine, reducing heart rate to 90 ± 8.0 beats/min, 5 to 10 min after morphine (4 mg/kg). Neostigmine (5 μg/kg/min) caused marked fasciculations and cardiac arrest in three animals when morphine was injected.

Discussion

Intravenous injection of morphine in cats, dogs and man produces an initial hypotensive response which is mainly due to histamine release (Feldberg and Paton, 1951; Evans *et al.*,

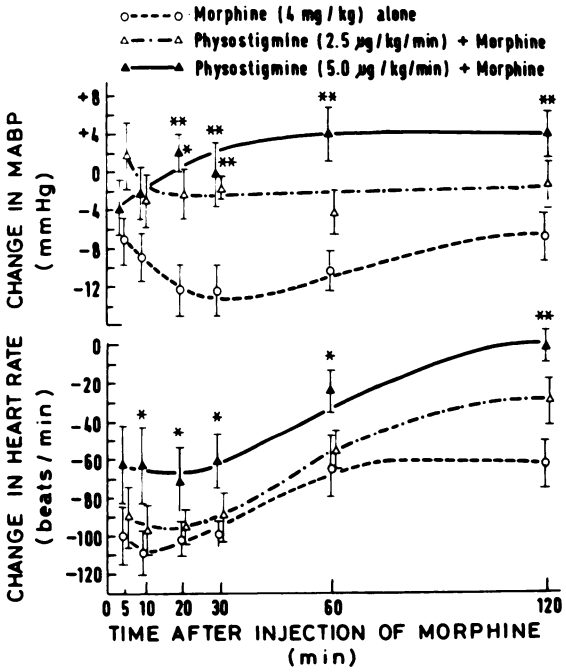

Fig. 2. The effect of physostigmine pretreatment on hypotension and bradycardia induced by morphine. MABP, mean arterial blood pressure.

TABLE 2

Effect of physostigmine on cardiovascular and respiratory parameters and plasma cholinesterase in the rabbit

Results are the mean values (S.E.M.) obtained 30 min after infusion of physostigmine or neostigmine. MABP, mean arterial blood pressure; Plasma Ch.E., plasma cholinesterase.

Treatment	N	MABP	Heart Rate	Respiration Rate	Blood pH	Pa_{CO_2}	Pa_{O_2}	Inhibition of Plasma Ch. E.
		mm Hg	*beats/min*	*min*		*mm Hg*	*mm Hg*	%
Predrug control	20	83.3 ± 2.3	272 ± 8	91 ± 6	7.40 ± 0.01	25.8 ± 1.2	100 ± 2	0
Physostigmine (25 μg/kg/min)	7	80.7 ± 2.2	268 ± 9	107 ± 9	7.46 ± 0.01*	18.9 ± 1.6*	107 ± 4	48.4 ± 2.8
Physostigmine (5 μg/kg/min)	8	87.4 ± 3.4	270 ± 10	110 ± 6*	7.46 ± 0.01*	19.8 ± 1.5*	108 ± 3**	55.3 ± 2.8
Neostigmine (2.5 μg/kg/min)	5	84.2 ± 2.8	248 ± 15	98 ± 8	7.41 ± 0.01	22.8 ± 1.8	104 ± 2	80.4 ± 4.8

* $P < .01$ compared to predrug control by paired *t* test; ** $P < .05$ compared to predrug control by paired *t* test.

TABLE 3

Influence of ATMN and hyoscine on the prevention of the respiratory depressant effect of morphine by physostigmine

Treatment	N	Respiration Rate	Blood pH	Pa_{CO_2}	Pa_{O_2}
Combined Control	24	96 ± 10	7.40 ± 0.01	25.1 ± 1.1	101 ± 4
Morphine (4 mg/kg, 30 min)	6	48 ± 8	7.24 ± 0.03	37.2 ± 1.6	84 ± 1.4
Physostigmine (5 μg/kg/min) + morphine (4 mg/kg, 30 min)	8	84 ± 7*	7.40 ± 0.03*	23.9 ± 2.3**	99 ± 4*
ATMN (0.5 mg/kg), Physostigmine (5 μg/kg/min) + morphine (4 mg/kg, 30 min)	6	87 ± 8*	7.41 ± 0.03*	23.1 ± 2.4**	98 ± 5*
Hyoscine (10 mg/kg), physostigmine (5 μg/kg/min) + morphine (4 mg/kg, 30 min)	4	45 ± 12	7.23 ± 0.03	37.9 ± 2.4	82 ± 4

Significantly different from morphine alone, * $P < .01$; ** $P < .001$.

1952; Grundy, 1971). This is usually followed by a longer lasting phase of hypotension which can be antagonized by specific opiate antagonists (Grundy, 1971). The site of the prolonged hypotensive response to opiates in dogs has been localized to the medulla oblongata and results from a reduction in sympathetic outflow to blood vessels (Laubie *et al.*, 1974).

Morphine causes bradycardia by a centrally induced increase in vagal tone (Laubie *et al.*, 1974). The respiratory depressant action of morphine is well known and has also been localized to the lower brain stem (Florez *et al.*, 1968).

In the present study in conscious rabbits, morphine (2 and 4 mg/kg i.v.) produced only the slowly developing long-lasting depressor response, presumably because rabbits are less sensitive to the vasodilator effects of histamine than are cats, dogs and humans (Rochae Silva, 1966). A larger dose of morphine (10 mg/kg i.v.) caused an initial pressor response and failed to produce consistent hypotension. This may have resulted from catecholamine release from the adrenal medulla (Grundy, 1971; Wallenstein, 1979), which was sufficient to overcome the centrally induced hypotensive response.

The magnitude of the fall in blood pressure induced by morphine (4 mg/kg) in the conscious rabbit was less than that reported in anesthetized cats and dogs for at least two reasons: firstly, the initial mean arterial blood pressure was only 80 to 85 mm Hg instead of 120 to 140 mm Hg in the dog (Laubie *et al.*, 1974) and cat (Evans *et al.*, 1952). Secondly, the cardiovascular reflexes remained intact in the rabbits in the present study, because of the absence of an anesthetic agent.

The hypotension induced by morphine (2 and 4 mg/kg) was preceded by a pronounced bradycardia, which, as in the dog, appeared to result from vagal stimulation, as it was antagonized by ATMN.

The cardiovascular effects of morphine were accompanied by respiratory depression, as indicated by a reduction in respiration rate, blood pH and Pa_{O_2} and a rise in Pa_{CO_2}.

In order to see whether the cardiovascular and respiratory depressant effects of morphine were due to an inhibition of the release of acetylcholine from neurons in the central nervous system, it was decided to administer a centrally acting anticholinesterase agent, physostigmine. Such a substance prevents the destruction of acetylcholine, thereby increasing its effect. This, in turn, should result in an antagonism of those effects of morphine that are associated with an interference of acetylcholine release.

Morphine itself can inhibit cholinesterase *in vitro*. However, Johannesson (1962) showed that the dose one needed to administer to rats to inhibit brain cholinesterase to any significant extent was greater than 500 mg/kg. Therefore, it is unlikely that morphine would either interfere with the action of physostigmine on the enzyme or modify its own effects on the respiratory and cardiovascular systems by inhibiting cholinesterase.

Because of its relatively short duration of action, physostigmine was administered as a continuous i.v. infusion to ensure that its effect would last as long as that of morphine *i.e.*, 2 to 3 hr. When infused at the rate of 2.5 or 5 μg/kg/min, physostigmine alone did not alter heart rate or blood pressure significantly, but it markedly stimulated respiration. This stimulant effect was antagonized by hyoscine but not by ATMN (Weinstock *et al.*, 1981), suggesting that physostigmine had caused a sufficient accumulation of acetylcholine to activate central muscarinic receptors influencing respiration but not the cardiovascular system. However, the hypotensive response to morphine (4 mg/kg/min) was completely prevented by pretreatment with physostigmine (2.5 μg/kg/min). The degree of respiratory depression was also considerably reduced. On the other hand, the peripherally acting anticholinesterase, neostigmine, did not antagonize any of these effects of morphine, but even potentiated the bradycardia.

In spite of the fact that physostigmine too might be expected to potentiate the bradycardia induced by morphine through inhibition of cholinesterase in the heart, nevertheless, it reduced both the extent and duration of the bradycardia when given at a dose of 5 μg/kg/min. It therefore seems likely that the central effect of physostigmine, which results in stimulation of the sympathetic supply to the heart (Brezenhoff, 1973), can overcome the peripheral effect.

Hypercapnia can stimulate respiration and has been shown to increase the release of acetylcholine from chemosensitive areas in the medullary reticular formation (Metz, 1966). This respiratory stimulant effect of carbon dioxide can be antagonized by application of atropine to these medullary neurons (Dev and Loeschcke, 1979). Morphine depresses respiration by reducing the sensitivity of the respiratory center in the medulla to carbon dioxide (Florez *et al.*, 1968). The mechanism by which it does so is not clear, but it could result from an impairment of the release of acetylcholine in response to hypercapnia.

Pretreatment of rabbits with physostigmine (5 μg/kg/min) prevented almost completely the usual rise in Pa_{CO_2} and fall in pH and Pa_{O_2} that occur after morphine administration. The antagonism by physostigmine of the respiratory depressant effect of morphine was prevented by pretreatment with hyoscine, a centrally acting antimuscarinic agent, but not by ATMN which blocks only peripheral cholinergic receptors. Furthermore, neostigmine, which also acts almost exclusively on peripheral cholinesterase, failed to prevent respiratory depressant effect of morphine.

These data demonstrate that an elevation of brain acetylcho-

line by physostigmine can overcome the respiratory depressant action of morphine. They also support the suggestion that morphine depresses respiration by reducing the release of acetylcholine from neurons in the medulla.

The hypotension and bradycardia induced by morphine were also antagonized by physostigmine and therefore they too may result from impairment of cholinergic transmission at sites in the central nervous system.

The lack of inhibitory effect of physostigmine on analgesia induced by morphine confirms previous reports in other species (Weinstock, 1971; Bhargava and Way, 1972). This finding is not surprising since physostigmine and cholinergic agonists themselves have been shown to possess analgesic activity both in animals and man (Ireson, 1970; Pleuvry and Tobias, 1971; Sitaram *et al.*, 1977). It suggests that impairment of cholinergic transmission may not mediate the analgesic effect of morphine. This is further supported by the finding of Moroni *et al.* (1977, 1978) that a decrease in acetylcholine turnover by opiates and opioid peptides of the limbic system is not related to analgesic activity.

In view of our findings, it may be possible to design a dose regime of treatment of human subjects with a combination of morphine and physostigmine (by continuous infusion) which would maintain analgesia without concomitant respiratory depression.

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