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Effects of Administration of Phentonium Bromide on Opioid Withdrawal Syndrome in Rats

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Abstract

This study has tested whether phentonium bromide, a quaternary ammonium anti-muscarinic agent, could reverse the signs of precipitated opioid withdrawal.

Rats were treated with either saline or morphine for 4 days, after which half the rats received naloxone and half saline. Each animal also received one of four doses of phentonium bromide (0, 1, 3 and 9 mg kg^{-1}, i.p.). Administration of phentonium bromide in rats receiving naloxone after chronic morphine treatment reduced the intensity of withdrawal signs such as increased defecation or micturition, salivation and wet-dog shakes, and elevated the nociceptive threshold values.

The effects of administration of phentonium bromide might result from its anti-muscarinic activity interfering peripherally with the mechanisms involved in the regulation of the withdrawal symptoms. The use of this drug is thus suggested as a possible means of controlling some of the signs observed during the acute phase of opioid withdrawal in heroin addicts.

Morphine has often been reported to affect the synthesis and release of acetylcholine (Hano et al 1964; Large & Milton 1970; Sharkawi 1972) and increased acetylcholine turnover has been described during morphine-withdrawal syndrome in particular (Domino & Wilson 1973), and attempts to control the morphine-withdrawal syndrome by antagonism of the cholinergic system have also been reported (Collier et al 1972; Pinsky et al 1973; Hynes et al 1976). For example, administration of the anti-muscarinic, atropine to rats affected by the morphine-withdrawal syndrome significantly reduced the jumping, diarrhoea and teeth chattering normally seen in such animals (Collier et al 1972).

In another study, morphine-abstinent rats given high doses of atropine showed a biphasic effect with an initial worsening of abstinence symptomatology and then an amelioration of the syndrome (Pinsky et al 1973). However, high doses of atropine have also been reported to be ineffective against symptoms such as wet-dog shakes, loss of body weight and piloerection, which are controlled by the cholinomimetic pilocarpine (Hynes et al 1976). It is possible that atropine treatment is not ideal for controlling morphine withdrawal behaviour because it is not selective in blocking central and peripheral withdrawal signs, because this drug can pass the blood–brain barrier.

Because blockade of morphine effects, e.g. reduction of delayed nociceptive reactions and the restoring of gastrointestinal transit, can be induced by quaternary narcotic antagonists acting peripherally (Bianchi et al 1982), it possible that some withdrawal signs could be controlled by a drug with a nitrogen quaternary group having peripheral activity.

Because of the close association between the development of morphine abstinence symptoms and activation of the cholinergic system reported in the literature (Brown & Taylor 1995), the current study was undertaken to investigate the effects of the anti-muscarinic phentonium bromide (Fig. 1), which presumably does not pass through the blood–brain barrier. Because this compound has been reported to affect salivation (Della Bella et al 1968; Azzolini et al 1970), gastrointestinal motility (Della Bella et al 1968; Moroni & Frigerio 1977), urinary bladder tone (Milani et al 1986) and pain-threshold levels (Ferrari et al 1968; Benelli & Santini 1974). Its effects on several opioid withdrawal signs have been examined.

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Materials and Methods

Experimental protocol and procedures were performed according to the regulations of Italian law: D.L. no. 116, 27/01/1992 and with the approval of the local University Committee on Laboratory animals.

Male Sprague–Dawley Rats, 200 ± 10 g, were obtained from Charles River (Calco). Morphine was obtained from S.A.L.A.R.S. (Como, Italy), naloxone from Sigma (Milan, Italy) and phentonium bromide (Ulcesium) from Zambon (Bresso, Milan, Italy). The drugs were dissolved in saline solution.

The scheme used for treatment with saline, morphine, naloxone, morphine plus naloxone, and phentonium bromide is depicted in Fig. 2. Rats were treated with either saline or morphine. Morphine was administered intraperitoneally (three injections each day at 150-min intervals) for four days, in doses of 9, 16 and 25 mg kg^{-1} (1st day); 25, 25 and 50 mg kg^{-1} (2nd day), 50, 50 and 50 mg kg^{-1} (3rd day) and 50, 50 and 100 mg kg^{-1} (4th day) while the other animals received saline. Half the rats in each group received naloxone, which was given at a dose of 30 mg kg^{-1}, intraperitoneally, 180 min after last morphine injection (naloxone and morphine + naloxone groups) the other half of the rats received saline only (morphine and control groups). Phentonium bromide (0, 1, 3 or 9 mg kg^{-1}) was administered intraperitoneally 60 min after the last administration of morphine to controls, morphine,

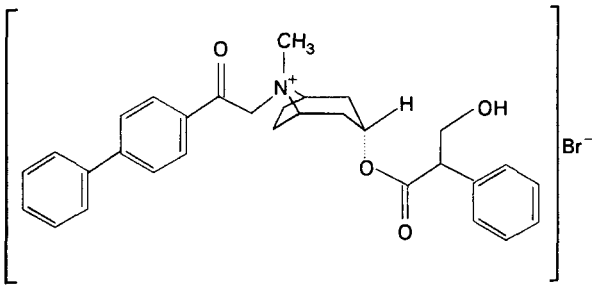

FIG. 1. The structural formula of phentonium bromide.

naloxone, and morphine+naloxone to other animals. The groups (controls, morphine, naloxone, morphine+naloxone) receiving only saline solution were indicated as dose 0 of phentonium bromide. The animals were initially randomly divided into two groups (90 animals receiving morphine and 64 animals not receiving morphine). The morphine group was larger because previous experience showed that morphine treatment resulted in approximately 30% mortality. Before treatment with phentonium bromide, a second randomization was used to select 32 animals for each of the four final groups.

The animals were placed in plastic cylinders (50 × 18 cm) over previously weighed filter-paper dishes. The signs observed and evaluated were: excretion of faeces by weighing the stool on the paper dishes; urine excretion by weighing the fluid content absorbed by the paper dishes after removal of faeces; rectal temperature by thermal probe; latency times by a tail-flick technique (Harris et al 1969) using equipment provided by Socrel, Milan (Italy). The tail-flick technique is based on the time between the exposure of the tail to heat (110°C) and the flick of the tail (latency time). A 20-s cut-off time was employed. Behavioural signs such as salivation, jumping and wet-dog shakes were also evaluated.

Hypersalivation, jumping and wet-dog shakes occurred only in the morphine+naloxone ± phentonium bromide groups and were evaluated either by scoring the intensity of the sign from 0 to 3 points (hypersalivation), by counting the number of events (jumping and wet-dog shakes) and also by counting the number of animals exhibiting the modified behaviour.

Excretion of faeces and urine, salivation, jumping and wet-dog shakes were monitored for 30 min after naloxone injection. Body temperature and pain threshold levels were evaluated 30 min after naloxone injection.

Data analysis

The weights of faeces and urine, rectal temperatures and latency were subjected to parametric statistical analysis. Firstly basal values (phentonium bromide dose 0) were compared among groups using the Tukey test to validate the experimental conditions (Armitage 1991). Secondly a simple analysis was performed within each group receiving saline, morphine, naloxone, morphine+naloxone by applying the Tukey test to the four doses of phentonium bromide (0, 1, 3 and 9 mg kg^{-1}). Hypersalivation, jumping and wet-dog shakes were evaluated only for groups receiving morphine + naloxone and four doses of phentonium bromide (0, 1, 3 and 9 mg kg^{-1}) and were analysed by the Tukey test and by the Armitage test (Armitage 1991).

Data on faeces and urine excretion, rectal temperature and latency times were also analysed by analysis of variance according to the original 2 × 2 × 4 factorial design (morphine, naloxone and four doses 0, 1, 3, 9 mg kg^{-1} of phentonium bromide) (Armitage 1991). In particular the P values for the F ratio are given for the effects of morphine, naloxone, phentonium bromide and for the interactions of morphine × naloxone, morphine × phentonium bromide, naloxone × phentonium bromide, and morphine × naloxone × phentonium bromide.

Statistical analysis was performed by use of SAS software, version 6·08 (SAS Institute, SAS Campus Drive, Cary, NC 27513). The components were considered to be statistically significant at the level of 5%.

Results and Discussion

Morphine and naloxone

Treatment of animals with morphine and naloxone increased the amount of faeces and urine excreted, reduced the rectal temperatures and latency times ($P < 0·05$ by Tukey test) and induced behaviour such as hypersalivation, jumping and wet-dog shakes not observed in animals not receiving both drugs.

Furthermore faecal and urinary excretion and rectal temperature appeared modified when evaluated in the factorial design as the main effect of morphine ($P < 0·0001$), naloxone ($P < 0·0001$ or $P < 0·02$), or as interaction of morphine with naloxone ($P < 0·0001$) (Table 1).

The effects observed in animals exposed to morphine and naloxone result from naloxone, which antagonizes the effects of morphine. The reported data confirm results described and extensively reviewed elsewhere (Martin & Jasinski 1977; Redmond & Krystal 1984; Bhargava 1994).

Phentonium bromide, morphine and naloxone

It is important to remark that the signs mentioned above were modified in different ways by administration of phentonium bromide, which prevented many of these precipitated withdrawal signs, as discussed in detail below.

Administration of phentonium bromide reduced faecal excretion ($P < 0·05$ by Tukey test) in controls, naloxone-, and morphine+naloxone-treated rats (Table 2) and exerted a reducing effect when it was evaluated as the main factor ($P < 0·0001$) in the analysis of variance (Table 1). The reducing effect exerted by phentonium bromide on faecal elimination seems to be independent of the presence or absence of the opioid or its antagonist and attributable to its anticholinergic activity, affecting gastrointestinal motility (Della Bella et al 1968; Moroni & Frigerio 1977).

Administration of phentonium bromide to animals receiving naloxone or morphine+naloxone reduced urinary excretion both as measured by the Tukey test ($P < 0·05$; Table 3) and as the main effect in the factorial analysis ($P < 0·0001$) (Table 1). The lowering effect exerted by phentonium bromide on urinary excretion is a result of its anti-muscarinic mechanism (Della Bella et al 1968), as is its capacity to control urinary incontinence (Milani et al 1986). In particular, phentonium bromide counteracted the effects of naloxone on urinary excretion, as is also apparent from interactions of naloxone × phentonium bromide ($P < 0·0044$), morphine × naloxone × phentonium bromide ($P < 0·0494$) (Table 1), where this physiological antagonism, exhibited by the two drugs naloxone and phen-

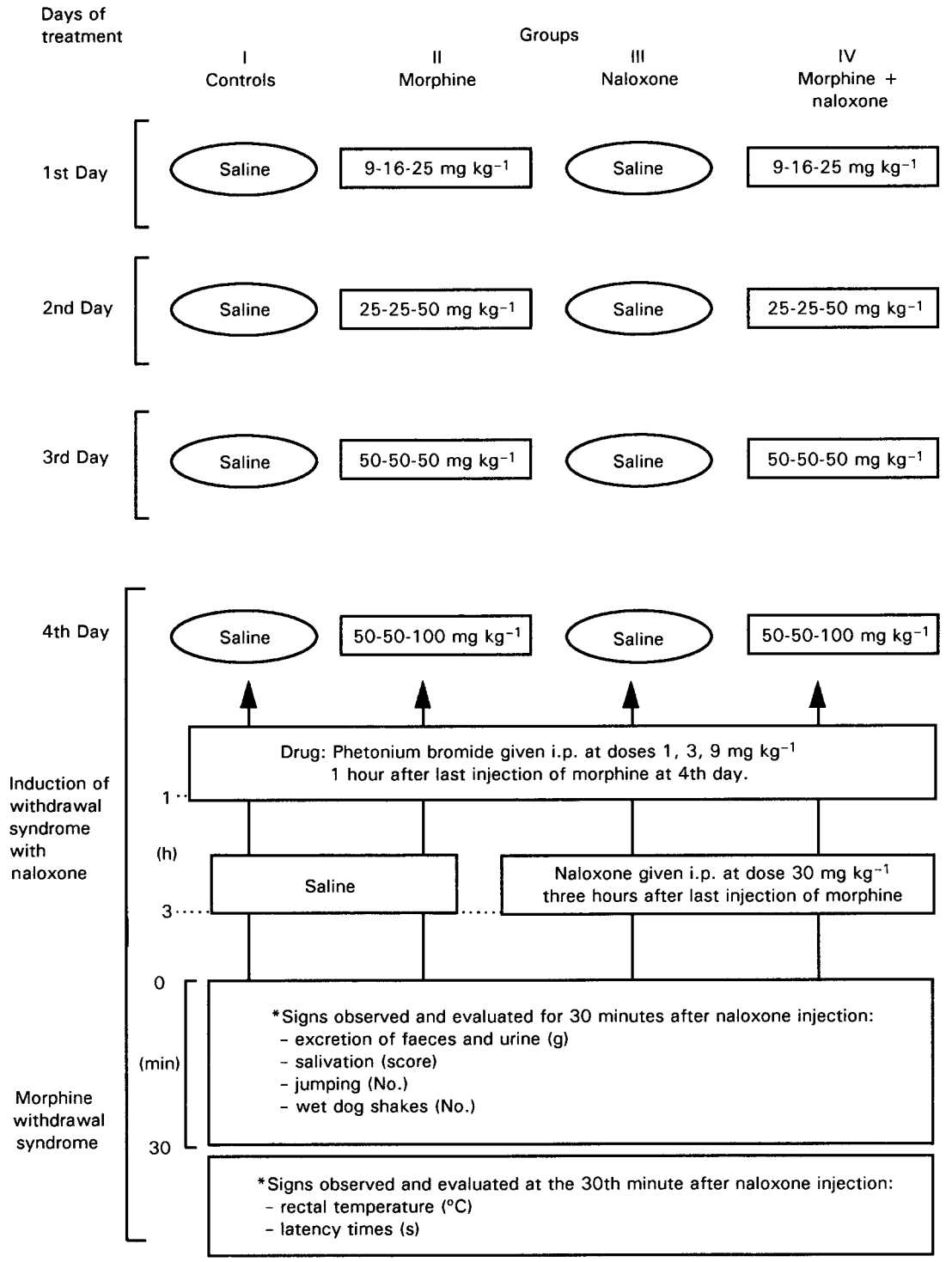

FIG. 2. Schematic diagram illustrating treatment with saline, morphine, naloxone, and morphine+naloxone, all plus phentonium bromide, and withdrawal signs. Withdrawal signs were monitored either 30 min after naloxone injection (rectal temperature, latency times) or for 30 min after naloxone injection (excretion of faeces and urine, salivation, jumping behaviour and wet-dog shakes).

tonium bromide, might be a result of the anti-muscarinic activity of phentonium bromide (Della Bella et al 1968) blocking the effect on micturition exhibited by acetylcholine released by naloxone (Redmond & Krystal 1984).

Administration of phentonium bromide to rats treated with saline, morphine or naloxone reduced rectal temperatures ($P < 0.05$ by Tukey test; Table 4). It was shown by analysis of variance that phentonium bromide had a highly significant ($P < 0.0001$) effect on rectal temperatures (Table 1). The temperature-reducing effect of this drug seems to be a consequence of a mechanism other than opioid activity, because it appears irrespective of whether the opioid or its antagonist is present or absent. Because this drug has been shown to block acetylcholine presynaptic receptors (Fann et al 1990), the

Table 1. Analysis of variance in a factorial design 2 × 2 × 4 ± morphine ± naloxone and four doses of phentonium bromide: single effects of the three variables morphine, naloxone, phentonium bromide and interactions morphine × naloxone, morphine × phentonium bromide, naloxone × phentonium bromide and morphine × naloxone × phentonium bromide related to levels of faecal excretion, urinary excretion, rectal temperature and latency times.

Source of variability	DF	Mean square	F	$P > F$
Faecal excretion				
Model	15	21·26	17·39	0·0001*
Morphine	1	126·37	103·34	0·0001*
Naloxone	1	76·07	62·21	0·0001*
Phentonium	3	10·71	8·76	0·0001*
Morphine × naloxone	1	75·70	61·90	0·0001*
Morphine × phentonium bromide	3	0·62	0·51	0·6755
Naloxone × phentonium bromide	3	0·78	0·64	0·5923
Morphine × naloxone × phentonium bromide	3	1·48	1·21	0·3101
Error	112	1·22		
Urinary excretion				
Model	15	11·01	15·56	0·0001*
Morphine	1	49·55	70·02	0·0001*
Naloxone	1	58·00	81·96	0·0001*
Phentonium bromide	3	4·85	6·85	0·0003*
Morphine × naloxone	1	24·70	34·90	0·0001*
Morphine × phentonium bromide	3	0·95	1·35	0·2634
Naloxone × phentonium bromide	3	3·27	4·62	0·0044*
Morphine × naloxone × phentonium bromide	3	1·91	2·70	0·0494*
Error	112	0·71		
Rectal temperature				
Model	15	9·42	33·84	0·0001*
Morphine	1	1·53	5·50	0·0208*
Naloxone	1	84·83	304·77	0·0001*
Phentonium bromide	3	6·48	23·27	0·0001*
Morphine × naloxone	1	31·60	113·54	0·0001*
Morphine × phentonium bromide	3	0·64	2·30	0·0808
Naloxone × phentonium bromide	3	0·50	1·79	0·1534
Morphine × naloxone × phentonium bromide	3	0·15	0·55	0·6476
Error	112	0·28		
Latency times				
Model	15	41·95	13·81	0·0001*
Morphine	1	36·13	11·89	0·0008*
Naloxone	1	1·05	0·35	0·5575
Phentonium bromide	3	127·02	41·82	0·0001*
Morphine × naloxone	1	4·96	1·63	0·2039
Morphine × phentonium bromide	3	31·10	10·24	0·0001*
Naloxone × phentonium bromide	3	6·79	2·24	0·0880
Morphine × naloxone × phentonium bromide	3	30·77	10·13	0·0001*
Error	112	3·04		

DF = degrees of freedom. * $P < 0·05$.

temperature-reducing effect exerted by phentonium bromide could be attributed to blockade of the M_2 inhibitory presynaptic receptor and to facilitated release of acetylcholine reducing body temperature (Redmond & Krystal 1984). This agrees with the observation that M_2 antagonists have been shown to cause hypothermia (Sen & Bhattacharya 1991).

Administration of phentonium bromide to saline-, morphine-, naloxone-, or morphine+naloxone-treated rats increased the latency times ($P < 0·05$ by Tukey test; Table 5) and significantly affected the latency times ($P < 0·0001$) when analysed as main effect by factorial design (Table 1). It is important to remark that the effect of phentonium bromide was independent of opioid activity because it appears in the presence or absence of the opioid or its antagonist and is attributable to the antinociceptive activity of phentonium, owing to its blocking of the nociceptive stimulation caused not only by acetylcholine but also by histamine, 5-hydroxytryptamine and bradykinin (Benelli & Santini 1974). This has also been associated with anti-spastic and anti-visceral pain effects in man (Ferrari et al 1968).

The treatment of rats with morphine and naloxone induced hypersalivation (Table 6). Administration of phentonium bromide significantly ($P < 0·05$ by Tukey test) reduced the intensity of this salivation because this drug is capable of blocking the salivary secretion through an anti-muscarinic mechanism (Della Bella et al 1968).

Jumping behaviour was observed in rats receiving morphine+naloxone (Table 6). Phentonium bromide treatment did not reduce the number of jumps.

Treatment with morphine+naloxone resulted in shaking behaviour (Table 6). Phentonium bromide reduced the number of shakes ($P < 0·05$ by Tukey test), presumably by blockade of

Table 2. Faecal excretion observed for 30 min for rats receiving saline, morphine, naloxone, and morphine+naloxone, all plus phentonium bromide.

A		Dose of phentonium bromide (mg kg^{-1}, i.p.)			
Group		0	1	3	9
Controls	I	1·053 ± 0·11	0·098 ± 0·06	0·018 ± 0·002	0·020 ± 0·002
Morphine	II	1·223 ± 0·27	0·744 ± 0·21	0·64 ± 0·29	0·343 ± 0·18
Naloxone	III	0·903 ± 0·17	0·265 ± 0·17	0·015 ± 0·001	0·010 ± 0·001
Morphine+naloxone	IV	5·376 ± 0·37	3·223 ± 0·51	3·581 ± 0·67	3·09 ± 0·671

Each value represents the mean ± s.e. of results from eight animals.

B Basal	Controls	Morphine	Naloxone	Morphine+naloxone
I compared with IV*	0 compared with 1*	0 compared with 9*	0 compared with 1*	0 compared with 9*
II compared with IV*	0 compared with 3*		0 compared with 3*	
III compared with IV*	0 compared with 9*		0 compared with 9*	

Significance levels $*P < 0·05$ by Tukey test.

Table 3. Urinary excretion observed for 30 min in rats receiving saline, morphine, naloxone, and morphine+naloxone, all plus phentonium bromide.

A		Dose of phentonium bromide (mg kg^{-1}, i.p.)			
Group		0	1	3	9
Controls	I	0·486 ± 0·1	0·271 ± 0·06	0·248 ± 0·08	0·183 ± 0·07
Morphine	II	0·651 ± 0·13	0·700 ± 0·29	0·544 ± 0·15	0·753 ± 0·08
Naloxone	III	1·109 ± 0·09	0·965 ± 0·16	0·233 ± 0·15	0·758 ± 0·17
Morphine+naloxone	IV	4·452 ± 0·67	2·809 ± 0·65	1·989 ± 0·35	2·301 ± 0·43

Each value represents the mean ± s.e. of results from eight animals.

B Basal	Controls	Naloxone	Morphine+naloxone
I compared with IV*	0 compared with 9*	0 compared with 3*	0 compared with 3*
II compared with IV*		1 compared with 3*	0 compared with 9*
III compared with IV*			

Significance levels $*P < 0·05$ by Tukey test.

Table 4. Rectal temperature observed at the 30th min for rats receiving saline, morphine, naloxone, and morphine+naloxone, all plus phentonium bromide.

A		Dose of phentonium bromide (mg kg^{-1}, i.p.)			
Group		0	1	3	9
Controls	I	37·90 ± 0·11	37·31 ± 0·18	37·16 ± 0·19	36·25 ± 0·13
Morphine	II	38·80 ± 0·10	38·40 ± 0·11	38·40 ± 0·18	37·75 ± 0·14
Naloxone	III	37·20 ± 0·15	36·61 ± 0·11	36·24 ± 0·19	36·04 ± 0·13
Morphine+naloxone	IV	35·95 ± 0·20	36·08 ± 0·19	35·55 ± 0·31	35·41 ± 0·36

Each value represents the mean ± s.e. of results from eight animals.

B Basal	Controls	Morphine	Naloxone	Morphine+naloxone
I compared with II*	0 compared with 3*	0 compared with 9*	0 compared with 1*	1 compared with 9*
I compared with III*	0 compared with 9*		0 compared with 3*	
I compared with IV*	1 compared with 9*		0 compared with 9*	
II compared with III*	3 compared with 9*			
II compared with IV*				
III compared with IV*				

Significance levels $*P < 0·05$ by Tukey test.

Table 5. Latency times observed at the 30th min for rats receiving saline, morphine, naloxone, and morphine+naloxone, all plus phentonium bromide.

A		Dose of phentonium bromide (mg kg^{-1}, i.p.)			
Group		0	1	3	9
Controls	I	4·16 ± 0·30	7·64 ± 0·55	8·66 ± 0·75	8·46 ± 0·45
Morphine	II	4·05 ± 0·22	5·80 ± 0·48	5·20 ± 0·31	8·05 ± 0·15
Naloxone	III	4·54 ± 0·27	8·58 ± 0·57	7·39 ± 0·45	7·58 ± 0·50
Morphine ± naloxone	IV	3·10 ± 0·10	3·15 ± 0·24	8·83 ± 0·75	10·33 ± 1·74

Each value represents the mean ± s.e. of results from eight animals.

B Basal	Controls	Morphine	Naloxone	Morphine+naloxone
I compared with IV*	0 compared with 1*	0 compared with 1*	0 compared with 1*	0 compared with 3*
II compared with IV*	0 compared with 3*	0 compared with 9*	0 compared with 3*	0 compared with 9*
III compared with IV*	0 compared with 9*	1 compared with 9*	0 compared with 9*	1 compared with 3*
		3 compared with 9*		1 compared with 9*

Significance levels $*P < 0·05$ by Tukey test.

Table 6. Salivation (score), jumping and wet-dog shakes (number of events) observed for 30 min in animals receiving morphine+naloxone and intraperitoneal phentonium bromide.

	Behaviour		
Treatment	Behaviour Salivation	Jumps	Wet-dog shakes
Morphine+naloxone	2·50 ± 0·38	9·88 ± 3·81	2·13 ± 0·30
Morphine+naloxone+1 mg kg^{-1} phentonium bromide	2·00 ± 0·42	8·88 ± 3·01	1·38 ± 0·53
Morphine+naloxone+3 mg kg^{-1} phentonium bromide	0·75 ± 0·37*	11·75 ± 4·24	1·13 ± 0·64
Morphine+naloxone+9 mg kg^{-1} phentonium bromide	0·75 ± 0·41*	9·64 ± 2·85	0·75 ± 0·41*

Each value represents the mean ± s.e. of results from eight animals. $*P < 0·05$, significant compared with morphine+naloxone (Tukey test).

muscarinic receptors mediating 5-hydroxytryptamine metabolism (Turski et al 1984) involved in wet-dog shaking behaviour (Kruszewska & Langwinski 1983; Cowan 1993).

Furthermore, a high percentage of the animals showing hypersalivation (87·5%), jumping (75%) and wet-dog shaking (75%) behaviour were observed to be in the group receiving morphine+naloxone. The administration of phentonium bromide to rats receiving combined morphine and naloxone significantly reduced the percentage of animals showing hypersalivation ($P < 0·05$) and wet-dog shaking ($P < 0·05$) behaviour, as evaluated by the Armitage test (χ^2 for slope).

Conclusions

Phentonium bromide has been shown to be capable of controlling several signs of morphine withdrawal; this activity might be linked to its anti-muscarinic properties (Della Bella et al 1968).

It is important to remark that opioid withdrawal signs are affected differently by anti-cholinergics and by cholinomimetics. Atropine-like compounds seem to be inactive in controlling some deprivation signs in rats, whereas cholinergic agents seem to reduce wet-dog shaking behaviour and to increase diarrhoea (Hynes et al 1976). Anti-muscarinic and anti-nicotinic compounds increase jumping behaviour whereas cholinergic drugs reduce this behaviour in mice (Brase et al 1974). In other reports the autonomic symptoms were ex-acerbated by atropine and blocked by physostigmine, whereas the non-autonomic symptoms were reduced by atropine and made worse by physostigmine (Bhargava & Way 1972; Jhamandas et al 1973). There is a clear involvement of the central and peripheral cholinergic systems in opioid abstinence expressions.

The use of phentonium bromide, a compound acting peripherally, might have the advantage of not interfering with central acetylcholine muscarinic receptors the activation of which appears to be necessary for the control of some signs of morphine-withdrawal syndrome, as is demonstrated by direct or indirect cholinomimetic administration. Furthermore, the intact activity of the central cholinergic system might be beneficial for the control of heroin addicts (Rupreht & Dworacek 1983–1984).

As reported in this paper, several symptoms of morphine deprivation can be controlled by use of peripherally acting drugs, as demonstrated by phentonium bromide. The concomitant administration of a centrally acting agent, e.g. a cholinomimetic or α_2-receptor agonist (Redmond & Krystal 1984) associated with phentonium bromide could be more effective in suppressing withdrawal signs.

Furthermore the doses used in this experimental model are close to those administered in therapy in man for anti-muscarinic, anti-spasmodic purposes; thus phentonium bromide might be effective in suppressing some acute withdrawal symptoms in heroin addicts.

Acknowledgements

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