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THE HYPERALGESIC ACTION OF BARBITURATES IN MICE

BY

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Since the introduction of the barbiturates there has been a difference of opinion as to whether these compounds have an analgesic action. While Keats & Beecher (1950) believe that small doses of pentobarbitone are analgesic in clinical pain, workers using experimental pain techniques in man and animals have been unable to demonstrate any significant analgesic action of barbiturates (Andrews & Workman, 1941; Smith, D'Amour & D'Amour, 1943; Wolff & MacDonald, 1944; Hart & Weaver, 1948). Recently it has been shown, using experimental pain techniques in man, that the barbiturates are actually hyperalgesic (Clutton-Brock, 1961; Dundee, 1960), and may cause an increased sensitivity to pain.

The work presented here confirms that barbiturates have an hyperalgesic action and shows that this property is not shared by chloral hydrate, paraldehyde, glutethimide, methylpentynol or hydroxydione.

METHODS

An electrical method was used for estimating the degree of analgesia in mice. In principle, the method was the same as that described previously (Neal & Robson, 1964). A rectangular-wave electronic stimulator was used, the output of which was led to a pair of electrodes with a 250-kΩ resistor in series. This allowed the 0- to 100-V range on the stimulator to be used, and to some extent reduced the effect of any change in resistance of the mouse tail. In fact, preliminary experiments with thiopentone showed that no change in skin resistance occurred at dose-levels which produced a marked hyperalgesic effect. The electrodes were specially shaped to fit the mouse tail and electrode jelly was used to ensure good electrical contact (Neal & Robson, 1964). The approximate voltage pain-threshold was determined for each mouse by giving a series of three shocks starting at 50 V and increasing by 5-V increments, until the mouse squeaked at least twice out of the three times. Rectangular-wave pulses were used of 100 msec duration and with a frequency of 0.5 shock/sec. This " threshold voltage " refers to the voltage setting of the stimulator and not to the actual voltage across the mouse tail which is much smaller (approximately 10 to 20 V).

To estimate the presence or absence of analgesia the mice were given a series of twenty shocks at 20 V above their threshold voltage (stimulating voltage). The number of times a mouse did not squeak out of twenty (negative response) was taken to be proportional to the degree of analgesia present. The mean negative response of about fifteen mice was obtained in most experiments. The stimulus of 20 V above threshold voltage was found to give control values of about 5. Thus results in the range 0 to 5 represented hyperalgesia while results in the range 5 to 20 represented an analgesic effect. In most experiments the mice were tested 10, 30, 60 and 90 min after administration of the drug.

The mean negative response from mice treated with drugs was compared with that obtained from controls. Tests for statistical significance were carried out as described previously (Neal & Robson, 1964). The drugs

used were: methohexitone sodium (Brietal), thiopentone sodium (Intraval), pentobarbitone sodium (Nembutal), phenobarbitone sodium (Roche), methylpentynol (Oblivon), glutethimide (Ciba), hydroxydione sodium succinate (sodium 21-hydroxypregnane-3,20-dione succinate; Viadril), bemigride (Megimide), morphine tartrate and pethidine hydrochloride. Chloral hydrate was administered as a 2% w/v solution in 0.9% saline, glutethimide as a suspension in 0.9% saline containing 0.05% w/v Tween 80, and paraldehyde as a 4% w/v solution in 0.9% saline.

All injections were intraperitoneal unless otherwise stated.

RESULTS

A summary of all the results is given in Tables 1 and 2.

Hyperalgesic effect of barbiturates

Methohexitone. In doses of 5 and 10 mg/kg, intravenously, this drug produced a significant hyperalgesic effect at 2 min. After 10 min this effect had virtually disappeared. A dose of 10 mg/kg given by slow intravenous injection produced ataxia but not loss of consciousness.

Thiopentone. This drug produced an hyperalgesic effect when injected intraperitoneally (Fig. 1). The magnitude of this effect appeared to increase with the dose given. However,

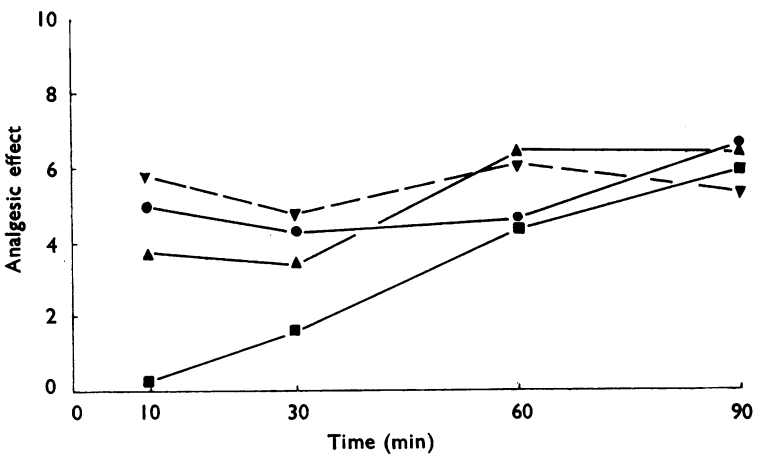

Fig. 1. Hyperalgesic effect of thiopentone. Doses of 7.5 and 15 mg/kg, intraperitoneally, produce no significant effect, but increasing the dose to 30 mg/kg produces a hyperalgesic effect which is significant at 10 and 30 min. Ordinate, analgesic effect (mean negative response); abscissa, time in minutes. ●, Control ; ▼, thiopentone (7.5 mg/kg) ; ▲, thiopentone (15 mg/kg); and ■, thiopentone (30 mg/kg).

although this trend was evident, it was only the highest dose (30 mg/kg) which produced a significant hyperalgesic effect. This was maximal at 10 min. A dose of 15 mg/kg produced a slight hyperalgesic effect which was, however, not significant. Reducing the dose to 7.5 mg/kg gave a response very similar to the control.

Pentobarbitone. This drug (30 mg/kg) produced a significant hyperalgesic effect at 10 min, but, unlike the effect of the same dose of thiopentone, the effect was not significant after 30 min.

Phenobarbitone. This drug produced a long-lasting hyperalgesic effect when given in high doses (Table 1). At 60 mg/kg the hyperalgesic effect was significant only at 10 min, but increasing the dose to 110 and 160 mg/kg produced an hyperalgesic effect which remained practically unchanged for the duration of the experiment (2 hr).

TABLE 1

HYPERALGESIC ACTION OF BARBITURATES

Controls (last row) were with 0·9% saline. I.v., intravenous. I.p., intraperitoneal. Probability values (*P*) are given in parentheses

Drug	Route	Dose (mg/kg)	Negative response at times (min) after injection					
			2	10	30	60	90	120
Methohexitone sodium	I.v.	5	2·08 (<0·02)	4·00	4·50	5·42	6·34	
		10	0·10 (<0·001)	2·10	4·30	4·10	4·60	
Thiopentone sodium	I.p.	7·5		5·8	4·73	6·07	5·27	
		15		3·78	3·39	6·3	6·39	
		30		0·27 (<0·001)	1·67 (<0·001)	4·33	5·93	
Pentobarbitone sodium	I.p.	30		1·13 (<0·001)	3·66	5·66	4·93	
Phenobarbitone sodium	I.p.	60		3·0 (<0·005)	2·86	3·86	5·00	6·15
		110		2·8 (<0·05)	0·7 (<0·001)	0·7 (<0·001)	1·2 (<0·001)	1·1 (<0·01)
		160		0·5 (<0·001)	0 (<0·001)	0 (<0·001)	0 (<0·001)	0 (<0·001)
Saline	I.p.	0·2 ml.		4·87	4·20	4·67	6·60	4·60
	I.v.	0·05 ml.	4·66	2·25	4·66	6·16	7·66	

Antagonism of analgesics by barbiturates

Thiopentone, pentobarbitone and phenobarbitone antagonized the analgesic effect of morphine and pethidine. Because of its transient action, methohexitone was not used in these experiments.

Antagonism of morphine and pethidine by thiopentone

The analgesic action of morphine (5 mg/kg) was completely abolished by simultaneous administration of thiopentone (30 mg/kg) (Fig. 2). A smaller dose of thiopentone (15 mg/kg) prevented the onset of morphine analgesia at 10 min, but after 30 min there was no significant reduction in the analgesic effect of morphine.

The injection of thiopentone (30 mg/kg) 30 min after the administration of morphine (10 mg/kg) completely abolished the analgesia which had been produced by morphine (Fig. 3). The same effect was obtained when the analgesia was produced by pethidine (20 mg/kg) (Fig. 3). Thus thiopentone will antagonize established analgesia produced by morphine or pethidine as well as prevent the development of analgesia when the analgesic drug and thiopentone are administered simultaneously.

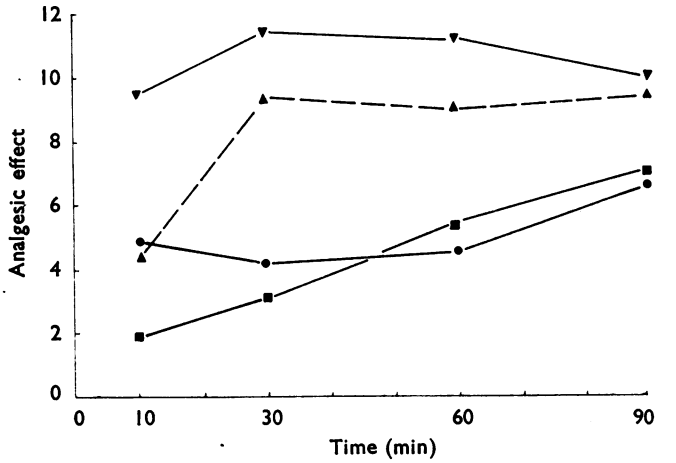

Fig. 2. Antagonism of morphine by thiopentone. The analgesic action of morphine (5 mg/kg, intraperitoneally) is completely abolished by simultaneous administration of thiopentone (30 mg/kg, intraperitoneally). Reducing the dose of thiopentone to 15 mg/kg delays the onset of the analgesic effect of morphine but after 30 min the hyperalgesic action of thiopentone is no longer significant. ●, Control; ▼, morphine (5 mg/kg) ; ▲, morphine and thiopentone (15 mg/kg) ; and ■, morphine and thiopentone (30 mg/kg).

Antagonism of morphine by pentobarbitone

This was very similar to the antagonism of morphine by thiopentone. The analgesic action of morphine (5 mg/kg) was initially abolished by the simultaneous administration of pentobarbitone (30 mg/kg). However, after 60 min there was no significant difference from the controls, which had received morphine only. Pentobarbitone (15 mg/kg), unlike the same dose of thiopentone, did not significantly antagonize the analgesic action of morphine (5 mg/kg).

Antagonism of morphine by phenobarbitone

Phenobarbitone (110 mg/kg) completely abolished the analgesic action of morphine (5 mg/kg) for the 2 hr of the experiment.

Antagonism of the hyperalgesic action of phenobarbitone by bemigride

The injection of bemigride (80 mg/kg), 30 min after the combined administration of morphine (5 mg/kg) and phenobarbitone (110 mg/kg), produced a level of analgesia which was not significantly different from that obtained in mice treated with morphine only (Fig. 4). Thus bemigride appeared to antagonize the hyperalgesic action of phenobarbitone. A control experiment using bemigride only (80 mg/kg) could not be performed as this dose quickly killed the mice which were unprotected by barbiturates. A nonconvulsive dose of bemigride (10 mg/kg) did not produce a significant increase in the mean negative response when compared with controls. However, when bemigride (80 mg/kg) was injected into mice which had been treated 30 min previously with chloral hydrate (250 mg/kg), the analgesic effect of the latter drug was markedly potentiated. Thus it would seem that bemigride in large doses can produce an " analgesic effect."

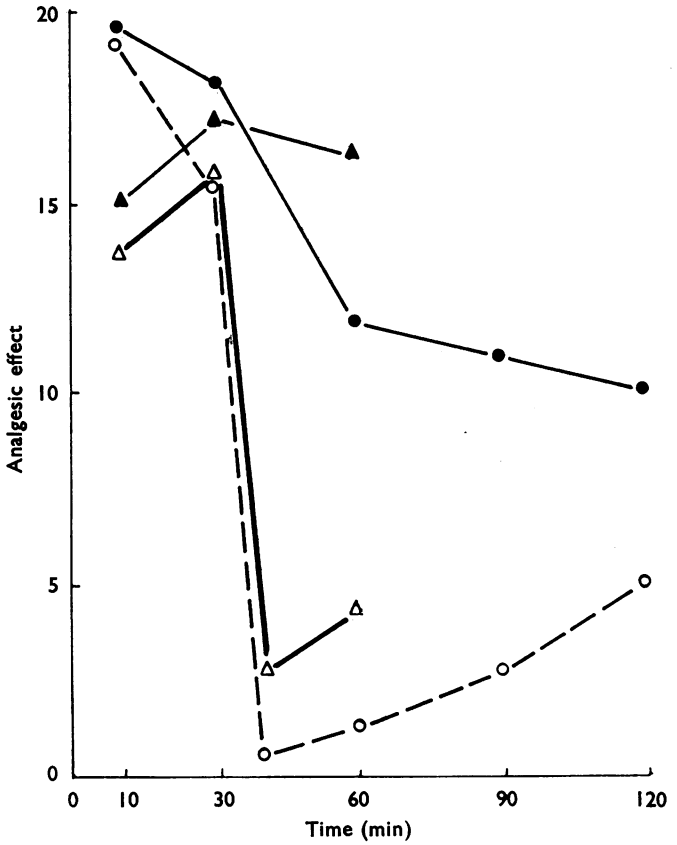

Fig. 3. Effect of thiopentone (30 mg/kg, intraperitoneally) on established analgesia produced by morphine (10 mg/kg, intraperitoneally) and pethidine (20 mg/kg, intraperitoneally). Thiopentone injected at 30 min abolishes the analgesic effect of both morphine and pethidine. ●, Pethidine (20 mg/kg); ○, pethidine (20 mg/kg) and thiopentone (30 mg/kg) injected at 30 min ; ▲, morphine (10 mg/kg) ; and △, morphine (10 mg/kg) and thiopentone (30 mg/kg) injected at 30 min.

Non-barbiturate drugs

Glutethimide. When injected intraperitoneally in doses of 50 and 100 mg/kg, this drug produced no hyperalgesic or analgesic effects (Table 2), although the higher of these doses produced severe ataxia.

Methylpentynol. When injected intraperitoneally in doses of 100 and 200 mg/kg, this drug did not produce any hyperalgesic effect. In fact, with the latter dose, a significant though short-lasting analgesic effect was produced (Table 2).

Chloral hydrate. This drug, in a dose of 125 mg/kg injected intraperitoneally, did not cause sleep or ataxia but did produce a slight analgesic effect (Table 2). Increasing the dose to 250 mg/kg produced sleep within 10 min. Although the mice could be gently placed on their side, they were not anaesthetized as stimulation caused them to move around. This dose produced a significant analgesic effect. In the mouse, chloral hydrate seems to be about 1/70th as active as morphine in producing analgesia.

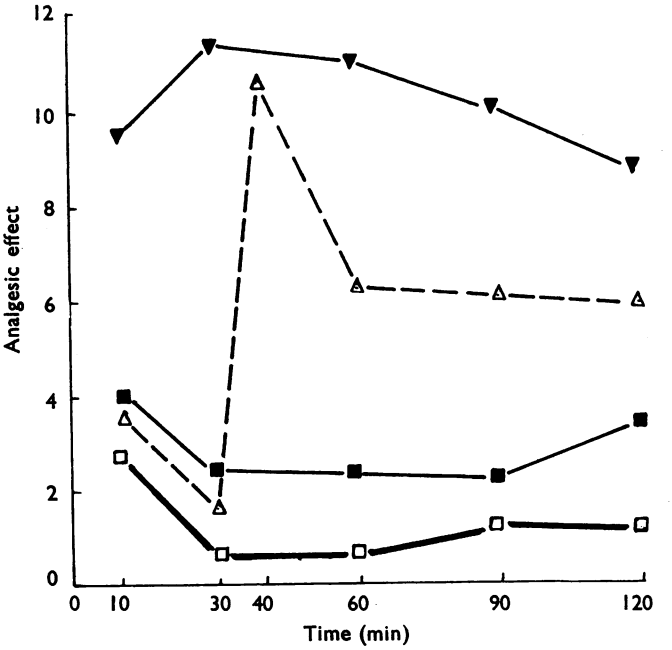

Fig. 4. Antagonism of the hyperalgesic action of phenobarbitone by bemigride. The injection of bemigride (80 mg/kg, intraperitoneally) 30 min after the combined administration of morphine (5 mg/kg, intraperitoneally) and phenobarbitone (110 mg/kg, intraperitoneally) produces a level of analgesia which is not significantly different from that obtained in mice treated with morphine only. ▼, Morphine (5 mg/kg); ■, morphine and phenobarbitone (110 mg/kg); □, phenobarbitone (110 mg/kg); and △, morphine (5 mg/kg) and phenobarbitone (110 mg/kg) and bemigride (80 mg/kg) injected at 30 min.

TABLE 2

SUMMARY OF RESULTS OBTAINED WITH NON-BARBITURATES (COMPARED WITH MORPHINE)

Probability values (*P*) are given in parentheses

Drug	Dose (mg/kg)	Negative response at times (min) after injection					Relative potency
		10	30	60	90	120	
Morphine tartrate	5	9·6 (<0·001)	11·5 (<0·001)	11·2 (<0·001)	10·1 (<0·001)	8·9 (<0·001)	100
Hydroxydione	20	7·2	9·46 (<0·01)	9·1 (<0·02)			18
Methyl-pentynol	100	7·5	6·4	6·9	5·7	6·5	1·9
	200	4	9·6 (<0·01)	9·4 (<0·01)	10	8·0	
Chloral hydrate	125	6·7	6·7 (<0·02)	7·7 (<0·01)	6·8	7·2 (<0·05)	1·4
	250	5·16	6·6 (<0·05)	7·9 (<0·02)	9·5 (<0·1)	9·9 (<0·01)	
Paraldehyde	250	4·0	4·0	4·4	6·8	6·8	1·1
	500	7·2	7·8 (<0·05)	8·6 (<0·02)	10·6	12·4	
Glutethimide	50	4·3	6·1	7·6	6·7	6·0	0
	100	3·7	3·8	5·75	7·55	7·8	

Paraldehyde. A dose of 250 mg/kg of this drug injected intraperitoneally produced no obvious effects. 500 mg/kg of paraldehyde produced ataxia within a few minutes and subsequently a gradually increasing analgesic effect was observed (Table 2). In the mouse, paraldehyde possesses about 1/90th of the analgesic activity of morphine.

Hydroxydione. Apart from the short-acting barbiturates, hydroxydione is the only intravenous anaesthetic used clinically in man. The results were interesting because, in contrast to the barbiturate anaesthetics, a subanaesthetic dose of hydroxydione (20 mg/kg) produced a significant analgesic effect after 30 min.

DISCUSSION

Significance of results

The electrical method of analgesimetry described has been found satisfactory for detecting both analgesic and hyperalgesic effects. Using this method, it has been shown that methohexitone, thiopentone, pentobarbitone and phenobarbitone all possess hyperalgesic properties in mice. This confirms the work of Dundee (1960) and Clutton-Brock (1961), both of whom demonstrated the hyperalgesic action of barbiturates in man, using pressure on the tibia as a source of experimentally induced pain. In the past many workers have tried to demonstrate that barbiturates possess analgesic properties. It is now clear why their efforts consistently met with failure. The barbiturates possess no analgesic properties in subanaesthetic doses. On the contrary, increasing the dose of barbiturate eventually produces an hyperalgesic effect. This effect has been demonstrated with four representative barbiturates and is, therefore, presumably an action common to all the barbiturates used clinically. However, in view of the " analgesic action " of certain stimulant drugs, it may be that the barbiturates with a convulsant action are not hyperalgesic, and may even produce an analgesic effect. This is at present being investigated.

Although all the drugs investigated possess similar pharmacological properties, it was only the barbiturates which were found to be hyperalgesic. It is interesting that glutethimide, which is structurally related to the barbiturates, has neither analgesic nor hyperalgesic properties. The remaining non-barbiturates, chloral hydrate, paraldehyde, methylpentynol and hydroxydione, all produce analgesia in high doses.

While the hyperalgesic action of the barbiturates becomes progressively greater as central nervous depression increases, the opposite occurs with chloral hydrate, paraldehyde, methylpentynol and hydroxydione. With the latter drugs increase in central nervous depression eventually causes the development of analgesia. Presumably this difference in effect is due to these drugs having different sites of action in the central nervous system.

The antagonism of the hyperalgesic action of phenobarbitone by bemigride was accompanied by the expected behavioural changes, namely a considerable reduction in ataxia and sedation. It seems that the antagonism of the hyperalgesic effect of phenobarbitone by bemigride is due to a direct " analgesic action " of bemigride, which is sufficiently powerful to overcome the hyperalgesic effect of phenobarbitone. This " analgesic action " of bemigride is not altogether surprising, as it has been shown that other centrally acting stimulants such as amphetamine and dexamphetamine are also capable of producing an " analgesic " effect (Goetzl, Burrill & Ivy, 1943, 1944; Burrill, Goetzl & Ivy, 1944).

The mode of action of hyperalgesics

The way in which barbiturates increase the sensitivity to pain is unknown. Hagbarth & Højeberg (1957) demonstrated that enhancement of evoked afferent discharge to the sensory cortex in man was induced by barbiturate anaesthesia. Thus it is tempting to suggest that the postoperative restlessness and excitement which sometimes occurs after barbiturate anaesthesia is somehow due to increased afferent discharge in the sensory cortex.

SUMMARY

1. The hyperalgesic effect of four barbiturates was estimated by an electrical method in which shocks were administered to mice through electrodes attached to their tails.

2. Methohexitone, thiopentone, pentobarbitone and phenobarbitone possess hyperalgesic properties in the mouse.

3. These barbiturates antagonized the analgesic effects of morphine and of pethidine.

4. Chloral hydrate, glutethimide, paraldehyde, methylpentynol and hydroxydione had no hyperalgesic effect. These compounds, with the exception of glutethimide, at high doses produced significant analgesia insufficient to cause anaesthesia.

5. The hyperalgesic action of phenobarbitone was antagonized by bemigride. This drug potentiates the analgesic action of chloral hydrate, hence antagonism of barbiturate hyperalgesia is due to a direct " analgesic action " of bemigride.

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REFERENCES

ANDREWS, H. L. & WORKMAN, W. (1941). Pain threshold measurements in the dog. *J. Pharmacol. exp. Ther.*, **73**, 99–103.

BURRILL, D. Y., GOETZL, F. R. & IVY, A. C. (1944). The pain threshold rising effects of amphetamine. *J. dent. Res.*, **23**, 337–344.

CLUTTON-BROCK, J. (1961). Pain and the barbiturates. *Anaesthesia*, **16**, 80–88.

DUNDEE, J. W. (1960). Alterations in response to somatic pain associated with anaesthesia. II. The effect of thiopentone and pentobarbitone. *Brit. J. Anaesth.*, **32**, 407–414.

GOETZL, F. R., BURRILL, D. J. & IVY, A. C. (1943). Analgesic effect of dextroamphetamine alone and in combination with morphine sulphate. *Fed. Proc.*, **2**, 16.

GOETZL, F. R., BURRILL, D. J. & IVY, A. C. (1944). The analgesic effect of morphine alone and in combination with dextroamphetamine. *Proc. Soc. exp. Biol. (N.Y.)*, **55**, 248–250.

HAGBARTH, K. E. & HØJEBERG, S. (1957). Evidence for subcortical regulation of the afferent discharge to the somatic sensory cortex in man. *Nature (Lond.)*, **179**, 526–527.

HART, E. R. & WEAVER, O. M. (1948). The analgesic and hypnotic actions of barbiturates. *Anesthesiology*, **9**, 276–280.

KEATS, A. S. & BEECHER, H. K. (1950). Pain relief with hypnotic doses of barbiturates and a hypothesis. *J. Pharmacol. exp. Ther.*, **100**, 1–13.

NEAI, M. J. & ROBSON, J. M. (1964). The measurement of analgesic properties of inhalation drugs given in sub-anaesthetic concentrations. *Brit. J. Pharmacol.*, **22**, 590–595.

SMITH, D. L., D'AMOUR, M. C. & D'AMOUR, F. E. (1943). The analgesic properties of certain drugs and drug combinations. *J. Pharmacol. exp. Ther.*, **77**, 184–193.

WOLFF, G. & MACDONALD, A. D. (1944). The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *J. Pharmacol. exp. Ther.*, **80**, 300–307.