Interactions of methocarbamol with morphine and aspirin

Methocarbamol is a centrally acting skeletal muscle relaxant which, in clinically used doses, displays little or no sedative activity. Its locus of action in reducing skeletal muscle spasm is generally thought to be the spinal cord (Truitt & Little, 1958). However, its ability to potentiate barbiturate-induced sleeping times (Truitt & Little, 1958) suggests that it also produces effects at supraspinal levels. A number of clinical reports have indicated that in patients treated with methocarbamol, requirements for analgesic therapy are reduced (Hudgins, 1959; Feinman & Sherman, 1961; Truitt, Morgan & Nachman, 1961). While inhibition of skeletal muscle spasm may reduce pain in its own right, it was thought that methocarbamol might possess analgesic activity itself, or alternatively, might potentiate the analgesic activity of other drugs.

In the present experiments, the interactions of methocarbamol with morphine and aspirin have been studied in analgesic tests with mice. Behavioural effects were also noted and a gross assessment of motor reflexes was obtained by eliciting a withdrawal reflex from a hind paw.

Analgesic activity was assessed by the hot plate method (Eddy & Leimbach, 1953), and the abolition of writhing after the intraperitoneal injection of phenylquinone (Hendershot & Forsaith, 1959). Complete abolition of writhing for a period of 30 min after phenylquinone was taken as the end-point for analgesia. In both tests analgesic activity was measured 30 min after the injection of the drugs and ED50 values were calculated using the method of Litchfield & Wilcoxon (1949).

Morphine sulphate was dissolved in distilled water and injected subcutaneously. Aspirin was injected intraperitoneally as its sodium salt. This was prepared immediately before its injection by dissolving aspirin in an aqueous solution containing the calculated quantity of sodium citrate to form sodium acetylsalicylate. Methocarbamol was dissolved in a 25% (*v*/*v*) solution of polyethylene glycol 300 (Shell) in distilled water and injected intraperitoneally. Control experiments showed that the solvents themselves did not influence the effects obtained.

Table 1 shows ED50 values obtained in the two analgesic tests using methocarbamol, morphine and aspirin.

At the ED50 for methocarbamol, the mice were severely sedated. At doses of 150–200 mg/kg, i.p., sedation was apparent and breathing became laboured. At higher dose levels (200–300 mg/kg, i.p.) the animals became cyanotic, while at the highest dose of methocarbamol used (400 mg/kg, i.p.) approximately 50% of the mice lost their righting reflex. No loss of withdrawal reflexes was apparent at this high dose level. It would therefore appear that the "analgesia" produced by metho-

Table 1. ED50 *values by the hot plate and writhing tests*

Drug	ED50 (mg/kg) Hot plate		Writhing	
	ED50	No. of mice	ED50	No. of mice
Methocarbamol (i.p.)	218·5 (240·4–198·6)*	120	262 (284–240)	100
Morphine sulphate (s.c.)	8·0 (9·9–6·4)	160	0·85 (0·98–0·74)	120
Acetylsalicylic acid (i.p.)	>500	200	210 (237–186)	140
Morphine sulphate (s.c.) + methocarbamol (i.p.) (50 mg/kg)	4·2 (5·1–3·5)	150	0·51 (0·61–0·43)	150
Acetylsalicylic acid (i.p.) + methocarbamol (i.p.) (50 mg/kg)	>500	120	128 (154–106)	100

* The figures in parentheses are the 95% confidence limits.

carbamol at the ED50 level was non-specific and resulted from the general depressant action of the drug at this dose level.

Aspirin at doses up to 500 mg/kg, i.p., produced little change in behaviour and no abolition of withdrawal reflexes. At doses of 200 mg/kg, i.p., slight stimulant activity was apparent, while at the highest doses used a quietening effect was produced. No satisfactory ED50 value could be obtained using the hot plate analgesic test; this confirms previous reports (Collier, 1964).

Morphine in doses of 2·5–15 mg/kg, s.c., produced a sedative action in mice, while at higher doses (15–20 mg/kg, s.c.) stimulation, as evinced by ceaseless movements, was apparent. The stimulant action of high doses of morphine in mice has been noted previously by Schaumann (1958) and other workers. At doses greater than 5 mg/kg, s.c. of morphine the Straub tail effect was noted. No abolition of withdrawal reflexes occurred in the dose range of morphine used.

In the presence of 50 mg/kg of methocarbamol, a dose which produced no behavioural or "analgesic" actions itself, the analgesic ED50 values for both morphine and aspirin were significantly reduced (Table 1). This may, in part, explain the reduced requirements for analgesic therapy in patients treated concurrently with methocarbamol and analgesics.

The potentiation of the analgesic action of aspirin in the presence of methocarbamol was not associated with any noticeable behavioural effects other than those seen with aspirin alone. However, in the presence of methocarbamol, the stimulant action of morphine was apparent at lower doses (5–10 mg/kg) than those required to produce this effect in animals treated with morphine alone. In animals treated with methocarbamol (50 mg/kg, i.p.) the Straub response to morphine (1–20 mg/kg, s.c.) was absent, and at doses of morphine greater than 3 mg/kg, s.c., withdrawal responses were abolished in more than 50% of the animals, an effect which was absent at the highest doses of morphine and methocarbamol used in the experiments when the drugs were given alone.

Both morphine and methocarbamol are known to block polysynaptic reflex activity in the spinal cord (Martin, 1963; Truitt & Little, 1958). Summation or mutual enhancement of this effect with the two drugs might explain the abolition of the withdrawal reflexes and of the Straub tail response which is thought to be of spinal reflex origin (Liemdorfer, 1948; Bibley, Salem & Grossman, 1960).

The ability of methocarbamol to potentiate the analgesic actions of both aspirin and morphine and to enhance the actions of the latter drug as a stimulant and spinal reflex depressant, suggests that specific interactions at the cellular level are unlikely. A major pathway involved in the metabolism and excretion of morphine, aspirin and methocarbamol is their conjugation with glucuronic acid. Competition between methocarbamol and aspirin or morphine for gluconuride formation might therefore be expected to enhance blood levels of the drugs, and this could explain the actions observed in the present experiments. This possibility is supported by the findings of Truitt, Morgan & Nachman (1961) who showed that plasma salicylate and methocarbamol levels in patients were significantly raised when the drugs were given in combination rather than separately.

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Studies on the subcellular distribution of [^{3}H]reserpine

It is well established that reserpine can deplete tissues of their catecholamine content. Since existing chemical methods could not detect reserpine in the tissues at a time when the amine content was still quite low, a "hit and run" or irreversible damage hypothesis was formulated to explain reserpine's mechanism of action. However, the development of [^{3}H]reserpine enabled workers (Shepard, Tsein & others, 1958; Maggiolo & Haley, 1964) to demonstrate trace quantities for up to five days post injection. Evidence is available indicating that the depletion induced by reserpine *in vivo* is, in all probability, due to a blockade of amine uptake by the adrenergic nerve granules. (Bertler, Hillarp & Rosengren, 1961; Stitzel & Lundborg, 1967). It is possible, therefore, that the subcellular site of action of reserpine may coincide with a similar subcellular binding of this depleting agent. The present experiments were conducted to test this hypothesis.

Male Swiss-Webster mice, 18–20 g, were injected with [^{3}H]reserpine, 600 μg/kg (approximately 155 μCi/kg) intravenously. The animals were killed by a blow on the head either 15 or 60 min post injection, the hearts removed and homogenized in an ice bath with a Teflon pestle, in 0·25M sucrose containing 0·005M phosphate buffer, pH 7·4 and 0·001 M $MgCl_2$. Nuclear and mitochondrial fractions were obtained by centrifuging in the cold for 10 min at 600 and 8000 *g*, respectively. The 8000 *g* supernatant was then spun at 105,000 *g* for 60 min in a Spinco model L ultracentrifuge to provide a microsomal and a high speed supernatant fraction. Each fraction was analysed for its [^{3}H]reserpine content by a modification of the method of Manara (1967). All sediments were resuspended twice in 10 volumes of acetone. The acetone was evaporated under a stream of nitrogen and the dried material was redissolved in chloroform. Ten volumes of chloroform were added to the high speed supernatant fraction, shaken for 15 min and then centrifuged at 600 *g* for 10 min to

Table 1. *Subcellular distribution of* [^{3}H] *reserpine in the mouse heart at* 15 *and* 60 *min periods after injection*

Fraction	15 min*	60 min*	% decrease
Nuclear	385 ± 23	143 ± 10	64
Mitochondrial	86 ± 12	30 ± 2	65
Microsomal	49 ± 3	19 ± 1	61
Supernatant	17 ± 3	8 ± 2	55

* Each value represents the mean content of [^{3}H]reserpine (ng/g) ± s.e. The means are based upon at least 10 experiments.