Potentiation of the actions of pentobarbitone by nikethamide

The analeptic drug nikethamide is still used as a respiratory stimulant in intoxication with central depressants (Goodman & Gilman, 1965). We have observed that nikethamide does not antagonize the action of pentobarbitone in mice, but in fact significantly prolongs the pentobarbitone-induced sleeping time.

Male mice (15–25 g) were injected with sodium pentobarbitone (45 mg/kg, i.p.). Sleeping time was measured as the time between loss and return of righting reflex. In a second group of animals, nikethamide (25 mg/kg) was injected intravenously 10 min after the barbiturate. Sleeping times are recorded in Table 1. Nikethamide significantly prolonged the sleeping time. There are two possible mechanisms for the potentiation of the action of sodium pentobarbitone by nikethamide. The analeptic or its metabolites could act as a depressant in mice, or nikethamide could inhibit the metabolism of the barbiturate.

The former proposition does not seem likely since nikethamide produced no signs of depression when injected into mice of the same strain, rather it increased spontaneous movement. To test the second possibility, mice were pretreated with SKF 525A (diethylaminoethyl diphenylpropylacetate). This drug inhibits microsomal enzymes which metabolize barbiturate drugs (Brodie, 1956). Mice pretreated with 3 mg/kg SKF 525A slept five times longer than amimals given pentobarbitone alone. The dose of sodium pentobarbitone was reduced in order to induce a sleeping time approximately equivalent to that of the previous experiments. The results of this experiment are also recorded in Table 1. Nikethamide significantly reduced sleeping time when microsomal enzymes were inhibited. If a single dose of nikethamide was injected into normal mice 24 h before an experiment, sleeping time was shortened, this was presumably due to the known action of nikethamide in causing enzyme induction (Kato & Chiesara, 1962).

Table 1. *Prolongation of pentobarbitone sleeping time by nikethamide in mice untreated and treated with SKF 525 A (3 mg/kg orally)*

Treatment	Sleeping time to pentobarbitone 45 mg/kg, i.p. alone	Sleeping time to pentobarbitone 30 mg/kg, i.p. after SKF 525 A
Saline 0·1 ml/10 g	12·75 ± 2·2 (10)*	13·0 ± 1·7 (10)*
Nikethamide 25 mg/kg	22·6 ± 2·4 (10) ($P = 0·02$)	2·32 ± 0·54 (10) ($P = 0·001$)
Saline 0·1 ml/10 g	10·8 ± 1·0 (10)	21·9 ± 2·5 (10)
Nikethamide 25 mg/kg	17·6 ± 2·1 (10) ($P = 0·02$)	4·0 ± 0·08 (10) ($P = 0·001$)

* No. of observations.

We believe these experiments demonstrate that nikethamide prolongs barbiturate sleeping time by inhibition of those microsomal enzymes that in normal conditions metabolise barbiturates. In the presence of SKF 525 A nikethamide produces its well known stimulant action and shortens barbiturate sleeping time.

These experiments bear out that observations of Kato, Chiesara & Vassanelli (1963) that nikethamide inhibits microsomal enzymes *in vitro*, and also strongly suggest that nikethamide is contraindicated in cases of respiratory depression due to barbiturate intoxication.

It is of note that textbooks of pharmacology still report that nikethamide is used for barbiturate induced respiratory failure and commercial literature still recommends this drug as a stimulant in overdosage with sedative drugs. We suggest that in cases of barbiturate overdosage nikethamide may well delay recovery.

Department of Biological Sciences,
Ewell County Technical College,
Ewell, Surrey, England.

REGINA M. BOTTING
D. BURDEN

January 12, 1969

REFERENCES

BRODIE, B. B. (1956). *J. Pharm. Pharmac.*, **8**, 1–17.

GOODMAN, L. S. & GILMAN, A. (1965). *The Pharmacological Basis of Therapeutics*, 3rd edn, p. 351. London: Collier-Macmillan.

KATO, R., CHIESARA, E. & VASSANELLI, P. (1963). *Biochem. Pharmac.*, **13**, 69–83.

KATO, R. & CHIESARA, E. (1962). *Br. J. Pharmac. Chemother.*, **18**, 29–38.

Inhibition of nucleic acid polymerases by salicylate *in vitro*

The incorporation of labelled amino-acids into the proteins of rat isolated diaphragm and of microsomal preparations from rat liver is inhibited by salicylate in concentrations of 0·3 mM and above (Dawkins, Gould & Smith, 1966). The initiation and maintenance of protein synthesis requires RNA and DNA and we have therefore studied the effects of salicylate on the activities of nucleic acid polymerases prepared from rat liver.

Liver nuclei were isolated from male rats (300–450 g) of the Wistar strain by the method of Widnell & Tata (1964) with the following modifications. A 10% (w/v) homogenate was centrifuged at 700 *g* for 10 min. The pellet was suspended in 5 vol of 2·4 M sucrose containing 1 mM $MgCl_2$, centrifuged at 50,000 *g* for 1 h and the purified nuclear pellet washed twice in 0·25 M sucrose containing 1 mM $MgCl_2$, before final suspension. RNA polymerase activity was estimated at 17°, to minimize interference from ribonuclease, by measuring the incorporation of radioactivity from ATP-α^{32}P into RNA in a Beckman LS 200B liquid scintillation system, using glass fibre discs. The RNA product from the mixtures incubated at 17° and 37° had a DNA-like base composition [(G + C)/(A + U) = 0·75]. DNA polymerase was purified up to the ammonium sulphate fractionation stage (Mantsavinos, 1964) and desalted by dialysis. Calf thymus DNA was used as a primer and the activity measured by the incorporation of radioactivity from dATP- ^{3}H into DNA.

Salicylate, in concentrations of 3 mM and above, significantly decreased the incorporation of radioactive ATP into RNA in the rat liver preparation (Table 1). DNA polymerase activity is inhibited by salicylate concentrations of 1 mM and above (Table 2).

These preliminary observations suggest that salicylates may interfere with the biosynthesis of nucleic acids and hence of proteins. A further implication of the present results is that an inhibitory action of salicylates on nucleic acid biosynthesis may be one of the factors concerned in the teratogenic effects of the drugs. It has been reported that the injection of the drugs in pregnant rats and mice not only produces premature birth and foetal death (Eriksson & Larsson, 1968) but also several congenital malformations of the litters carried to full term (Warkany & Takacs, 1959; Larsson, Bostrom & Ericson, 1963).