Antagonism of analgesics by amine-depleting agents

SIR,—The antagonism of the analgesic effect of morphine in mice by reserpine was first reported by Schneider (1954) and confirmed more recently by Takagi, Takashima & Kimura (1964). The analgesic response of morphine in reserpinised mice could be returned to control levels by administration of 3,4-dihydroxyphenylalanine, 5 hydroxytryptophan or a combination of the two compounds (Takagi, & others, 1964). They suggested that a possible mechanism for this antagonism of morphine analgesia by reserpine might arise in the need for catecholamines and 5-hydroxytryptamine (5-HT) in the mediation of the action of morphine. The depletion of these amines by reserpine would be expected to result in the failure of morphine to produce analgesia.

The present investigation was undertaken to elucidate further the mechanism of reserpine antagonism of morphine and to discover whether reserpine also antagonised analgesics of the salicylate type.

Male albino mice (Harlan Industries), 18–22 g, were used, and analgesia measured by the ability of morphine or acetylsalicylic acid to antagonise the writhing response which was induced by the intraperitoneal injection of 10 ml/kg of 0·1% hydrochloric acid solution (Eckhardt, Cheplovitz, Lipa & Govier, 1958). ED50 values were calculated by the method of Litchfield & Wilcoxon (1949).

In Table 1 are shown the effects of reserpine, α-methyldopa, and α-methyl-*m*-tyrosine on the ED50 of morphine and acetylsalicylic acid. Reserpine significantly elevated the ED50 values of morphine and acetylsalicylic acid indicating an antagonism of their analgetic effects. Unexpectedly, neither α-methyldopa nor α-methyl-*m*-tyrosine altered the ED50 values of morphine or acetylsalicylic acid. Nor had α-methyldopa or α-methyl-*m*-tyrosine any effect on the writhing response by themselves.

TABLE 1. THE EFFECT OF VARIOUS AMINE DEPLETORS ON THE ANALGESIC RESPONSE TO MORPHINE AND ACETYLSALICYLIC ACID

Treatment	Route	Time[1]	Dose mg/kg	ED50[2] Morphine SO_4 mg/kg s.c.	ED50[2] Acetylsalicylic acid mg/kg s.c.
Control	—	—	—	0·89 (0·71–1·11)	49·5 (42·0–58·4)
Reserpine	s.c.	24	5	2·48 (2·20–2·81)	200·0 (160·0–250·0)
α-Methyldopa	i.p.	4	400	0·88 (0·67–1·16)	35·0 (28·7–42·7)
α-Methyl-*m*-tyrosine	i.v.	4	400	0·61 (0·41–0·91)	42·0 (36·9–47·9)
	i.v.	24	400	1·13 (0·96–1·32)	50·1 (38·6–65·6)

[1] Time of pretreatment
[2] ED50 (Confidence limits)

If depletion of catecholamines or 5-HT or both were responsible for the reserpine antagonism of morphine or acetylsalicylic acid analgesia, then both α-methyldopa (Smith, 1960) and α-methyl-*m*-tyrosine (Hess, Connamacher, Ozaki,& Udenfriend, 1961) which deplete catecholamines and 5-HT should also antagonise this analgesia. Since neither of these two brain amine-depleting agents significantly elevated the ED50 values of morphine or acetylsalicylic acid, it is conceivable that the antagonism of analgesia by reserpine is due to some intrinsic property of reserpine other than its effect on brain amines. This

conclusion is supported by earlier work which suggested that the reserpine antagonism of the anticonvulsant effect of diphenylhydantoin (Chen, Ensor & Bohner, 1954) was competitive and not the result of brain amine depletion (Gray, Rauh & Shanahan, 1963).

Biomedical Research Department, Pitman-Moore Division of The Dow Chemical Company Indianapolis, Indiana, U.S.A.
March 16, 1965

A. D. RUDZIK
J. H. MENNEAR

References

Chen, G., Ensor, C. R. & Bohner, B. (1954). *Proc. Soc. exp. Biol., N.Y.*, **86**, 507–510.
Eckhardt, E. T., Cheplovitz, F., Lipa, M. & Govier, W. M. (1958). *Ibid.*, **98**, 186–188.
Gray, W. D., Rauh, C. E. & Shanahan, R. W. (1963). *J. Pharmacol.*, **139**, 350–360.
Hess, S. M., Connamacher, R. H., Ozaki, M. & Udenfriend, S. (1961). *Ibid.*, **134**, 129–138.
Litchfield, J. T. & Wilcoxon, F. (1949). *Ibid.*, **96**, 99–113.
Schneider, J. (1954). *Proc. Soc. exp. Biol., N.Y.*, **87**, 614–615.
Smith, S. E. (1960). *Brit. J. Pharmacol.*, **15**, 319–327.
Takagi, H., Takashima, T. & Kimura, K. (1964). *Arch. int. Pharmacodyn.*, **149**, 484–492.

Rapid release of ^{3}H-metaraminol induced by combined treatment with protriptyline and reserpine

SIR,—Two different amine uptake and concentrating mechanisms of the adrenergic neurone have been demonstrated, namely, the amine transport mechanism of the cell membrane, "the cell membrane pump", and the uptake mechanism of the specific storage granules (Carlsson, Hillarp & Waldeck, 1962; Hamberger, Malmfors, Norberg & Sachs, 1964; Hillarp & Malmfors 1964; Malmfors, 1965; Carlsson & Waldeck, 1965a, b). Either of these mechanisms can be selectively blocked by drugs. Thus protriptyline and desipramine were found to block the former, reserpine and prenylamine the latter mechanism.

In the present investigation the effect of simultaneous blockade of the two mechanisms, or of either mechanism alone, was investigated, using ^{3}H-metaraminol as an indicator. Mice were given ^{3}H-metaraminol 0·02 mg/kg intravenously, followed after 15 min by protriptyline 10 mg/kg i.v., or reserpine 0·5 mg/kg i.v., or a mixture of both. The animals were killed 15 or 45 min after the administration of the inhibitors. Determination of ^{3}H-metaraminol in heart was performed as described earlier (Carlsson & Waldeck, 1965a). Given alone protriptyline or reserpine caused a moderate reduction of ^{3}H-metaraminol in the heart (Table 1). In combination, however, the two drugs caused a rapid and pronounced decrease of the amine. Within 15 min, 80% of the ^{3}H-metaraminol had disappeared, and 45 min after the drug mixture had been given only 5% was left. Preliminary experiments where reserpine was replaced by prenylamine gave essentially the same result.

Analogous results were obtained in experiments where the ^{3}H-metaraminol had been given 3 days before protriptyline and reserpine, alone or in combination.