VOLKMAN, P., KOHLI, J., GOLDBERG, L., CANNON, J. G. & LEE, T. (1977). *Proc. natn. Acad. Sci. U.S.A.*, **74**, 3602–3606.

WEINTAUB, W. & HOPFINGER, A. (1973). *J. Thor. Biol.*, **41**, 53–60.

WOODRUFF, G. N., WATLING, K. J., ANDREWS, C. D., POAT, J. A. & MCDERMED, J. D. (1977). *J. Pharm. Pharmac.*, **29**, 422–427.

The antagonism of the analgesic effect of dipyrone by L-dopa and its relation to brain amine concentrations

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Brain biogenic amines, particularly 5-HT and noradrenaline, have a major role in the mediation of the analgesic response (Montoya & Bardisa, 1970; Jauhari & Bapat, 1971). Most studies correlating analgesia and brain monoamines have used narcotic analgesics (see Sparkes & Spencer, 1971). The non-addictive antipyretic analgesics, however, have received less attention. Goerlitz & Frey (1972) reported that no significant change in the analgesic effect of amino-antipyrine was brought about by prior treatment with antagonists of 5-HT or catecholamines. However, Paalzow (1973), suggested that salicylates exerted at least part of their analgesic action by interference with catecholaminergic neurons.

In the present study, the oral median analgesic dose (AD50) of dipyrone (Novalgin, Hoechst), determined in Swiss albino mice using the hot plate technique (Woolfe & Macdonald, 1944) at 55° (±0·02°), was found to be 90·0 mg kg^{-1} orally, with fiducial limits 62·1–130·5 at $P = 0{\cdot}05$.

The hot plate reaction time (HPRT) was determined in 5 groups of animals, 30 mice each, given saline, dipyrone, 90·0, and 450 mg kg^{-1} by mouth, L-dopa, 100 mg kg^{-1} (i.p.), and dipyrone (90 mg kg^{-1}) simultaneously with L-dopa (100 mg kg^{-1}) at 30, 60, and 90 min after initial drug administration. The normal HPRT was taken as the mean of two determinations, 30 min and immediately before treatment.

To estimate the brain concentrations of 5-HT and noradrenaline, five groups of 24 mice were treated in the same way as for HPRT determination. The animals were killed 1 h after treatment, the whole brains of 4 mice were pooled and amine estimations made spectrophotofluorometrically using a modification of the method of Mead & Finger (1961). Chromatographic separation of the catecholamines ensured that dopamine did not interfere with the noradrenaline estimation.

L-Dopa, given alone, induced a transient increase in the HPRT 30 min after injection (Table 1). Dipyrone induced marked analgesia, which was maintained over 90 min. When dipyrone was given simultaneously with L-dopa, the combination induced a significant increase in the HPRT, which was maintained for 30 min.

Although dipyrone, 90 mg kg^{-1}, induced a 17% decrease in the concentration of 5-HT in the brain and a 28% decrease in that of noradrenaline the ratio of 5-HT: noradrenaline was significantly increased by about 16% from normal. The higher dose of dipyrone (450 mg kg^{-1}) induced a significant increase in 5-HT, without affecting the noradrenaline concentration of the brain. Accordingly, the ratio of 5-HT: noradrenaline was elevated by 23% above the control.

Treatment with L-dopa alone caused a marked increase in the noradrenaline concentration of the brain to the extent of 150% without significant change in the 5-HT concentration. The ratio of 5-HT: noradrenaline was significantly reduced by about 34%. The combination of dipyrone with L-dopa had a similar effect to L-dopa alone. Thus the noradrenaline concentration was significantly raised by 52% and though the 5-HT concentration was also raised by 30%, the value was not statistically significant. The ratio of 5-HT: noradrenaline was not significantly affected by the combined treatment.

The observed analgesia of dipyrone thus correlates more with its effect on the brain ratio of 5-HT: noradrenaline, rather than with its effect on the brain concentration of either amine. The higher dose of dipyrone, caused a greater rise in 5-HT concentration and consequently in the 5-HT: noradrenaline ratio. The concomitant administration of L-dopa lowered this ratio, and subsequently returned the sensitivity of the animals to the nociceptive thermal stimulus to normal.

The relation between brain amines and analgesia is controversial. Morphine analgesia has been shown to be antagonized by inhibitors of catecholamine biosynthesis (Verri, Graff & Carrado, 1967) or by depletors of 5-HT (Major & Pleuvry, 1971). The participation of 5-HT in the central mediation of morphine analgesia was shown by Goerlitz & Frey (1972), but this was disputed by Buxbaum, Yarbrough & Carter (1973) who furthermore reported potentiation of morphine analgesia by α-methyltyrosine. Morphine analgesia was shown to be dependent on the 5-HT: dopamine ratio (Pleuvry & Tobias, 1971) or on the 5-HT: noradrenaline ratio (Sparkes & Spencer, 1971). An increase in 5-HT would therefore promote analgesia, while a decrease would tend to antagonize the antinociceptive effect.

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Table 1. *The changes in the hot plate reaction time and in brain amine concentrations in mice following treatment by dipyrone, L-dopa and their combination.* (* $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$ *vs* pretreatment values or saline controls).

Treatment	Mean hot plate reaction time (s) (±s.e.m.)				Brain amine concentration 1 h after treatment (μg mg^{-1} fresh tissue)		
	Before treatment	After treatment (min) 30	60	90	5-HT	NA	5-HT: NA
Saline 100 ml kg^{-1} orally	27·8 ±1·8	27·7 ±1·9	28·1 ±1·5	28·5 ±1·5	0·59 ±0·01	0·54 ±0·03	1·09 ±0·04
Dipyrone 90 mg kg^{-1} orally	23·9 ±0·4	34·1** ±2·7	42·5*** ±4·3	46·6*** ±3·8	0·49 ±0·03*	0·39 ±0·02**	1·26 ±0·04**
Dipyrone 450 mg kg^{-1} orally	25·9 ±0·4	44·1*** ±2·7	52·9*** ±3·7	57·7*** ±4·3	0·67 ±0·01***	0·50 ±0·02	1·34 ±0·04***
L-Dopa 100 mg kg^{-1} i.p.	28·8 ±1·8	35·4* ±2·3	24·9 ±1·7	24·6 ±1·5	0·58 ±0·09	0·81 ±0·07**	0·72 ±0·07**
L-Dopa 100 mg kg^{-1} i.p. + Dipyrone 90 mg kg^{-1} orally	28·7 ±1·4	37·2** ±2·4	28·6 ±1·5	28·0 ±1·3	0·77 ±0·10	0·82 ±0·03***	0·94 ±0·09

Our present findings are in agreement with the work of Sparkes & Spencer (1971), indicating that the analgesic effect of dipyrone correlates with a rise in the steady state balance of brain ratio of 5-HT: noradrenaline. Dipyrone is likely to act through hypothalamic subcortical areas (Woodbury, 1971) where noradrenaline, but not dopamine, is likely to occur in high concentrations (Pscheidt, Morpurgo & Himwich, 1964; Hornykiewicz, 1966). L-Dopa, by increasing the noradrenaline concentration, tends to lower the ratio of 5-HT: noradrenaline and therefore antagonize the analgesic effect of dipyrone.

October 21, 1977

REFERENCES

BUXBAUM, D. M., YARBROUGH, G. G. & CARTER, M. E. (1973). *J. Pharmac. exp. Ther.*, **185**, 317–327.

GOERLITZ, B. D. & FREY, H. M. (1972). *Eur. J. Pharmac.*, **20**, 171–180.

HORNYKIEWICZ, O. (1966). *Pharmac. Rev.*, **18**, 925–964.

JAUHARI, A. C. & BAPAT, S. K. (1971). *Ind. J. Physiol. Pharmac.*, **15**, 21–26.

MAJOR, C. T. & PLEUVRY, B. J. (1971). *Br. J. Pharmac.*, **42**, 512–521.

MEAD, J. A. R. & FINGER, K. F. (1961). *Biochem. Pharmac.*, **6**, 52–53.

MONTOYA, G. & BARDISA, L. (1970). *Arch. Biol. med. Exp.*, **7**, 8–10.

PAALZOW, L. (1973). *Acta pharmac. tox.*, **32**, 11–25.

PLEUVRY, B. J. & TOBIAS, M. A. (1971). *Br. J. Pharmac.*, **43**, 706–714.

PSCHEIDT, G. R., MORPURGO, C. & HIMWICH, G. E. (1964). In *Comparative Neurochemistry*, pp. 401–412. Editor: Richter, D. Oxford: Pergamon.

SPARKES, C. G. & SPENCER, P. S. J. (1971). *Br. J. Pharmac.*, **42**, 230–241.

VERRI, R. A., GRAFF, F. G. & CARRADO, A. P. (1967). *J. Pharm. Pharmac.*, **19**, 264–265.

WOODBURY, D. M. (1971). In: *The Pharmacological basis of Therapeutics*, Editors: Goodman, L. S. & Gilman, A. pp. 314–348. New York: Macmillan.

WOOLFE, E. & MACDONALD, A. D. (1944). *J. Pharmac. exp. Ther.*, **80**, 300–310.