liposomes was also significant; 1.6 per cent of entrapped sodium was released after 6 hr from lecithin–cholesterol liposomes compared to 5.8 per cent from lecithin liposomes.

The molecular composition of biological membranes and the concentration of carbenoxolone are therefore both important in determining the effect of the drug on membrane permeability. Studies to determine the actual membrane concentration of carbenoxolone in different liposomal systems may give more information on its molecular interactions in lipid membranes.

Acknowledgements—Dr. S. Gottfried of Biorex Laboratories Ltd., London, England is thanked for the supply of sodium carbenoxolone and Professor D. V. Parke for his encouragement and interest in this project.

Department of Biochemistry,
University of Surrey,
Guildford,
Surrey GU2 5XH,
England

ANDREW M. SYMONS

REFERENCES

1. R. Doll, I. D. Hill, C. Hutton and D. J. Underwood, *Lancet* II, 793 (1962).
2. R. Doll, *Scot. med. J.* **9**, 183 (1964).
3. J. M. Robson and F. M. Sullivan (Eds), *A Symposium on Carbenoxolone Sodium.* Butterworths, London (1968).
4. J. H. Baron and F. M. Sullivan (Eds), *Carbenoxolone Sodium.* Butterworths, London (1970).
5. F. Avery Jones and F. M. Sullivan (Eds), *Carbenoxolone in Gastroenterology.* Butterworths, London (1972).
6. F. Avery Jones and D. V. Parke (Eds), *Fourth Symposium on Carbenoxolone.* Butterworths, London (1975).
7. B. Johnston, W. E. Lindup, J. S. Shillingford, M. Smith and D. V. Parke, in *Fourth symposium on Carbenoxolone* (Eds F. Avery Jones and D. V. Parke.) p. 3. Butterworths, London (1975).
8. M. Lipkin, in *Carbenoxolone sodium.* (Eds J. H. Baron and F. M. Sullivan) Butterworths, London (1970).
9. J. G. Spenney, G. Saccomani, H. L. Spitzer, M. Tomana and G. Sachs, *Archs Biochem. Biophys.* **161**, 456 (1974).
10. A. D. Bangham, M. M. Standish and J. C. Watkins, *J. molec. Biol.* **13**, 238 (1965).
11. G. B. Robinson, in *Biological membranes.* (Ed D. S. Parsons) p. 40. Clarendon Press, Oxford (1975).
12. D. Papahadjopoulos, *Biochim. biophys. Acta* **265**, 169 (1972).
13. M. A. Singer, *Biochem. Pharmac.* **23**, 2939 (1974).
14. R. B. Heap, A. M. Symons and J. C. Watkins, *Biochim. biophys. Acta.* **218**, 482 (1970).
15. D. Papahadjopoulos and N. Miller, *Biochim. biophys. Acta.* **135**, 624 (1967).
16. S. M. Johnson, *Biochem. Soc. Trans.* **3**, 160 (1975).

Biochemical Pharmacology, Vol. 25, pp. 1547–1548. Pergamon Press, 1976. Printed in Great Britain.

p-Chlorophenylalanine-induced enhancement of the effects of morphine on the adrenal medulla

(*Received* 23 *September* 1975; *accepted* 13 *November* 1975)

The actions of morphine on the adrenal medulla can be explained in large part by its combined direct and centrally mediated stimulation of the sympatho–adrenal axis [1–4]. Thus, acute administration of morphine results in adrenal catecholamine depletion and trans-synaptic induction of the catecholamine biosynthetic enzymes, tyrosine hydroxylase and dopamine β-hydroxylase, as well as increased formation of new storage vesicles [1,2]. Upon chronic administration, however, the ability of morphine to deplete catecholamines disappears and levels increase to supranormal values; this recovery is related directly to the stimulation-induced increases in catecholamine biosynthetic enzymes and storage vesicles [1]. However, there is evidence that other factors may operate in limiting both the degree to which morphine can deplete adrenal catecholamines and the degree to which enzyme induction can occur. Morphine even in large doses appears to be incapable of evoking the full degree of stimulation of which the sympatho–adrenal axis is capable, and no additional induction of adrenal tyrosine hydroxylase is evident at doses of morphine exceeding 40 mg/kg [1,2]. Mueller *et al.* [5] and Breese *et al.* [6] have shown that depletion of central serotonin with *p*-chlorophenylalanine (PCPA) can enhance the sympatho–adrenal effects of other stimulatory agents, such as insulin or amphetamine. Since chronic morphine enhances serotonin turnover [7,8], it is possible that sympatho–adrenal stimulation by morphine is limited in part by enhancement of serotonergic negative input in the brain-stem [9]. In the present study, the action of PCPA on chronic morphine-induced stimulation of the adrenal medulla has been examined.

Male Sprague–Dawley rats (Zivic–Miller) weighing 200–250 g were given morphine HCl subcutaneously twice daily as follows: 10 mg/kg for 2 days, followed by 40 mg/kg for 2 days, followed by 100 mg/kg thereafter. Controls received saline on the same schedule. After 1 week at the highest dose, saline- or morphine-treated rats received saline or PCPA methyl ester HCl (150 mg/kg, i.p.) once daily for 2 days and were killed 24 hr after the second PCPA injection; animals continued to receive morphine or saline concurrently with PCPA or saline and thus were killed 12 hr after the last injection of morphine.

Adrenals from the rats in the four groups (control, PCPA, morphine, PCPA plus morphine) were excised and each pair was homogenized in 2 ml of 0.15 M KCl. Aliquots (0.1 ml) were deproteinized with 1.9 ml of 3.5% perchloric acid, centrifuged at 26,000 *g* for 10 min, and the supernatant was analyzed for catecholamines by the trihydroxyindole method using an autoanalyzer [10]. Duplicate 0.2 ml aliquots of the homogenate were used for analysis of dopamine β-hydroxylase activity by the periodate oxidation method [11], using 10 μM[^{3}H]-tyramine as substrate and *p*-hydroxymercuribenzoate (optimal concentration, 0.5 mM) to inactivate endogenous inhibitors. The

remainder of the adrenal homogenate was centrifuged at 26,000 *g* for 10 min to sediment the catecholamine-containing storage vesicles, and the supernatant utilized for duplicate determinations of tyrosine hydroxylase activity by the method of Waymire *et al.* [12], with 0.1 mM [^{14}C]-tyrosine as substrate. Results are expressed as means ± standard errors, and levels of significance calculated by Student's *t*-test.

Morphine HCl was obtained from Merck, Sharp & Dohme; tyramine [G-^{3}H] (10 Ci/m-mole) and L-tyrosine [1-^{14}C] (10 mCi/m-mole) were purchased from New England Nuclear Corp., and *p*-chlorophenylalanine methyl ester HCl and *p*-hydroxymercuribenzoate from Sigma Chemical Co.

The administration of PCPA alone produced little or no alteration in adrenal catecholamines or tyrosine hydroxylase or dopamine β-hydroxylase activities (Fig. 1). Chronic morphine alone resulted in elevations in all three parameters ranging from 160 to 220 per cent of controls. In the morphine-dependent rats, doses of PCPA which in themselves had no effect produced a complete reversal of the morphine-induced increase in adrenal catecholamines (from 160 per cent of control to 80 per cent). PCPA also enhanced markedly the induction of tyrosine hydroxylase evoked by chronic morphine but caused only a small increase in the actions of chronic morphine on dopamine β-hydroxylase activity (not statistically significant compared to morphine alone).

These results indicate that PCPA enhances the stimulatory effect of morphine on the sympatho-adrenal axis. While chronic morphine alone evokes adrenal catecholamine secretion, the stimulation results also in induction of tyrosine hydroxylase and dopamine β-hydroxylase activities which can maintain normal or elevated amine levels despite accelerated turnover. The additional increment in morphine-induced sympatho-adrenal stimulation after PCPA produces a pattern similar to that after acute morphine administration, where massive secretion occurs to an extent which cannot be compensated by enzyme induction [1,2]; consequently, catecholamine levels fall, while the additional stimulation increases further the tyrosine hydroxylase activity. The equivocal changes in dopamine β-hydroxylase in rats given PCPA plus morphine vs morphine alone reflect two opposing processes: additional loss of soluble dopamine β-hydroxylase via increased exocytotic secretion [13,14] and trans-synaptic enzyme induction [1,2,13,14].

The data obtained in this study are consistent with the hypothesis that the degree of sympatho-adrenal stimulation by morphine is limited normally by negative input via central serotonergic neurons; thus, depletion of serotonin with PCPA results in an enhancement of the morphine-induced stimulation. This hypothesis is supported by the observations of Ho *et al.* [7] and Way *et al.* [8] that chronic morphine administration increases serotonin turnover in the mouse whole brain and in brainstem, an area which contains serotonergic neurons responsible for reducing sympatho-adrenal outflow [9]. The dual nature of morphine's actions on sympatho-adrenal activity thus contributes to the inability both to maintain catecholamine depletion and to induce tyrosine hydroxylase to the extent seen after other stimulatory agents which act by different mechanisms [15,16]. Only upon elimination of the serotonergic component with PCPA does the full sympatho-adrenal response to chronic morphine become evident.

Acknowledgements Supported by USPHS DA-00465. Dr. Slotkin is the recipient of Research Scientist Development Award K01-DA-00006 from the National Institute on Drug Abuse.

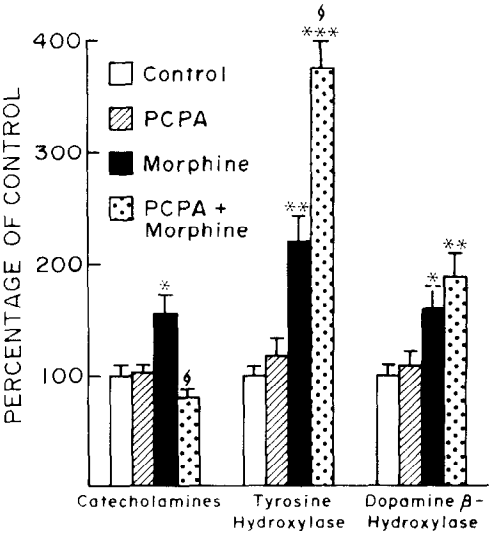

Fig. 1. Actions of *p*-chlorophenylalanine (PCPA) and morphine on rat adrenal catecholamines, tyrosine hydroxylase and dopamine β-hydroxylase. Data represent means ± standard errors of three to five animals. Controls values were: catecholamines, 12.6 ± 1.3 μg/gland; tyrosine hydroxylase, 8.42 ± 0.76 nmoles $^{14}CO_2$ evolved/hr/gland; dopamine β-hydroxylase, 1.57 ± 0.16 nmoles ^{3}H-octopamine formed/hr/gland. Key: (*) $P < 0.05$ vs control; (**) $P < 0.01$ vs control; (***) $P < 0.001$ vs control; and (§) $P < 0.005$ vs morphine.

Department of Physiology and Pharmacology
Duke University Medical Center, Durham, N.C. 27710, U.S.A.

THOMAS R. ANDERSON*
THEODORE A. SLOTKIN†

REFERENCES

1. T. R. Anderson and T. A. Slotkin, *Biochem. Pharmac.* **24,** 671 (1975).
2. T. R. Anderson and T. A. Slotkin, *Biochem. Pharmac.*, in press.
3. T. Yoshizaki, *Jap. J. Pharmac.* **23,** 695 (1973).
4. E. L. Way and F. H. Shen, in *Narcotic Drugs: Biochemical Pharmacology* (Ed. D. Clouet), p. 229. Plenum, New York (1971).
5. R. A. Mueller, R. D. Smith, G. R. Breese and B. R. Cooper, *Biochem. Pharmac.* **23** (suppl. 1), 128 (1974).
6. G. R. Breese, B. R. Cooper and R. A. Mueller, *Br. J. Pharmac. Chemother.* **52,** 307 (1974).
7. I. K. Ho, S. E. Lu, S. Stolman, H. H. Loh and E. L. Way, *J. Pharmac. exp. Ther.* **182,** 155 (1972).
8. E. L. Way, H. H. Loh and F. H. Shen, *Science, N.Y.* **162,** 1290 (1968).
9. A. Ito and S. M. Schanberg, *J. Pharmac. exp. Ther.* **181,** 65 (1972).
10. R. J. Merrills, *Analyt. Biochem.* **6,** 272 (1963).
11. S. Friedman and S. Kaufman, *J. biol. Chem.* **240,** 4763 (1965).
12. J. C. Waymire, R. Bjur and N. Weiner, *Analyt. Biochem.* **43,** 588 (1971).
13. T. A. Slotkin and N. Kirshner, *Biochem. Pharmac.* **22,** 205 (1973).
14. O. H. Viveros, L. Arqueros and N. Kirshner, *Science, N. Y.* **165,** 911 (1969).
15. T. A. Slotkin and F. J. Seidler, *Life Sci.* **16,** 1613 (1975).
16. F. J. Seidler and T. A. Slotkin, *Br. J. Pharmac. Chemother.* **56,** 201 (1976).

* Present address: School of Dentistry, University of North Carolina, Chapel Hill, N.C., U.S.A.
† Author to whom reprint requests should be addressed.