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# The stimulation of glycogenolysis and gluconeogenesis in isolated hepatocytes by opioid peptides

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The opioid agonists [leucine]enkephalin, [D-Ala$^2$,D-Leu$^5$]enkephalin and dynorphin-(1–13)-peptide, but not morphine, stimulated the conversion of [2-$^{14}$C]pyruvate into glucose and glycogenolysis when added directly to isolated hepatocytes. Naloxone produced a small but significant inhibition of both the basal and stimulated rate of incorporation of label into glucose but had no effect on the total glucose output by the cells. The effects of the opioid peptides were mediated by a cyclic AMP-independent mechanism.

It is clearly established that opiates exert considerable effects on glucose homoeostasis *in vivo*, since administration of morphine results in hyperglycaemia in a number of species (Borrison *et al.*, 1962; Feldberg & Gupta, 1974; Dey & Feldberg, 1975), whereas infusion of the morphine antagonist naloxone has been reported to decrease glucose production and utilization in the conscious dog (Werther *et al.*, 1981). Wong and co-workers have demonstrated that treatment of rats with morphine over a period of 6–24h results in an increase in the rate of glucose synthesis by liver slices prepared from the treated animal as a result of both an induction of a number of gluconeogenic enzymes and a suppression of pyruvate kinase (Wong *et al.*, 1978; Wong & Yeung, 1981). From these studies it is suggested that morphine may act directly on the liver to stimulate glucose synthesis. However, this interpretation is not unequivocal, as there is considerable evidence to suggest that the effects of morphine and other opiates on carbohydrate metabolism *in vivo* are mediated through opiate-sensitive receptors within the brain, with subsequent activation of the sympathetic nervous system and catecholamine release (Feldberg & Gupta, 1974; Dey & Feldberg, 1975). In addition, exogenous opiates may stimulate vasopressin release from the posterior pituitary (Weitzman *et al.*, 1977) and also act peripherally at the level of the pancreas to increase the secretion of insulin and glucagon (Ipp *et al.*, 1978; Green *et al.*, 1980; Reid & Yen, 1981).

The experiments in this study were designed to examine the effects of morphine and other opiate agonists on carbohydrate metabolism at the level of the isolated hepatocyte. Using this experimental system it is possible to distinguish between acute effects of the opiate exerted directly on the liver cell and secondary effects, which may be mediated by alterations in hormone release, thus eliminating many of the problems of interpretation inherent in the previous studies *in vivo*.

## Experimental

Hepatocytes were prepared from fed male Sprague–Dawley rats and incubated in Krebs–Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932) containing 1.5% (w/v) gelatin at 37°C as described previously (Chisholm *et al.*, 1983). The final incubation volume was 1.6ml of cells. A mixture of [2-$^{14}$C]pyruvate (sp. radioactivity 50mCi/mol) plus unlabelled lactate in a ratio of 1:2 was added as the gluconeogenic substrate to produce a final concentration of substrate of 5mM. Opioid peptides and other hormones were added as 100-fold concentrates dissolved in 0.9% NaCl containing 1mg of bovine serum albumin/ml. After a 15min incubation period, the rate of gluconeogenesis was estimated by the incorporation of [2-$^{14}$C]pyruvate into glucose as described previously (Chisholm *et al.*, 1983), and the total glucose output by the cells (gluconeogenesis plus glycogenolysis) determined by the measurement of the amount of glucose in the extracellular medium using glucose oxidase (Bergmeyer & Bernt, 1974). The rate of glycogenolysis was determined by measurement of the total glucose output in the absence of any gluconeogenic substrate. Cyclic AMP was determined as described previously (Chisholm *et al.*, 1983).

6-Ethyl-1-oxocyclazocine was a gift from Winthrop Laboratories (Surbiton-on-Thames, Surrey, U.K.), and naloxone was from Endo Laboratories (New York, NY, U.S.A.). Morphine sulphate was obtained from McCarthys (London, U.K.). All

other chemicals were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.) or BDH Chemicals (Poole, Dorset, U.K.).

Results are expressed as means ± S.E.M. for the numbers of different cell preparations given in parentheses. Statistical analysis of results was performed using a pooled $t$-test.

**Results and discussion**

The effect of a number of opioid agonists on the rate of conversion of [2-$^{14}$C]pyruvate into glucose and the total glucose output by the cells has been examined (Table 1). Addition of the classical $\mu$-receptor agonist, morphine, had no acute effect on the rates of either glucose synthesis or release by the cells at the final concentration of 15 $\mu$M or the higher concentration of 150 $\mu$M (results not shown). In contrast, addition of the opioid peptides [leucine]enkephalin and [D-Ala$^2$,D-Leu$^5$]enkephalin, which are believed to act via activation of $\delta$-receptors (Lord *et al.*, 1976), resulted in an 88% and 77% increase in the rates of both glucose formation from [2-$^{14}$C]pyruvate and glucose output respectively. Ethyloxocyclazocine, a non-peptide opiate agonist that binds preferentially to the $\kappa$-receptor (Lord *et al.*, 1977), also stimulated glucose production by the cells in some experiments, although this was not significant over the entire series ($P > 0.2$). However, addition of the peptide $\kappa$-agonist, dynorphin-(1–13)-peptide, produced a consistent significant increase in the activity of both pathways ($P < 0.001$). Also shown in Table 1 are the effects of maximal concentrations of glucagon (1 nM) and adrenaline (10 $\mu$M). There was no significant difference between the magnitude of the stimulation produced by any of the opioid peptides and that of glucagon or adrenaline on glucose release ($P > 0.2$), although [leucine]enkephalin proved to be more efficacious than adrenaline in stimulating the incorporation label into glucose ($P < 0.02$). The responses to [leucine]enkephalin were not additive with those of 1 nM-glucagon on either function. The rates of glucose synthesis from [2-$^{14}$C]pyruvate for control, [leucine]enkephalin, glucagon and [leucine]enkephalin-plus-glucagon were 23 ± 3, 34 ± 4, 37 ± 5 and 38 ± 6 nmol/h per mg wet wt. of cells respectively, and those of glucose output were 201 ± 39, 310 ± 40, 342 ± 34 and 348 ± 34 nmol/h per mg wet wt. of cells respectively ($n = 5$).

Table 2 shows the effect of the morphine anatagonist naloxone on the rates of conversion of [2-$^{14}$C]pyruvate into glucose and total glucose accumulation in the medium in both control cells and cells stimulated with [leucine]enkephalin. Naloxone had no effect on either the basal or stimulated rate of glucose output, although it significantly depressed the basal rate of incorporation of label into glucose ($P < 0.01$) and reduced the degree of stimulation by [leucine]enkephalin by 30%. The slight reduction in the basal rate of glucose synthesis correlates with the ability of naloxone infusion to decrease glucose production in the conscious dog despite a rise in plasma glucagon and no change in the plasma insulin concentrations (Werther *et al.*, 1981). This may indicate that the gluconeogenic pathway is under some degree of tonic control by opiate receptors. However, the possibility of some non-specific inhibitory action of naloxone at the level of one of the gluconeogenic enzymes cannot be excluded. The weak antagonism of the effect of [leucine]enkephalin by naloxone is in agreement with the known greater affinity of naloxone for $\mu$- versus $\delta$-receptors (Shaw *et al.*, 1982). In contrast with longer-term studies (Wong *et al.*, 1978; Wong & Yeung, 1981), the above data suggest that there is no direct involvement of $\mu$-type receptors in the acute regulation of hepatic carbohydrate metabolism and

Table 1. *Effect of opiate agonists, glucagon and adrenaline on the rate of conversion of [2-$^{14}$C]pyruvate to glucose and total glucose output in isolated hepatocytes*

The protocol was as described in the Experimental section. The hormones and peptides were added to the final concentrations indicated and the incubation carried out for 15 min. *$P < 0.01$ and **$P < 0.001$ for significance of difference from controls.

| Additions | Rate (nmol/h per mg wet wt. of cells) | |
|---|---|---|
| | Conversion of [2-$^{14}$C]pyruvate to glucose | Total glucose output |
| Vehicle | 17.3 ± 1.2 (12) | 131.6 ± 11.0 (12) |
| Morphine (15 $\mu$M) | 18.4 ± 1.1 (5) | 106.6 ± 11.0 (5) |
| [leucine]Enkephalin (15 $\mu$M) | 32.3 ± 2.4 (12)** | 249.3 ± 16.0 (12)** |
| [D-Ala$^2$,D-Leu$^5$]Enkephalin (15 $\mu$M) | 26.2 ± 1.7 (6)** | 218.9 ± 17.0 (6)** |
| Ethyloxocyclazocine (15 $\mu$M) | 22.2 ± 4.9 (6) | 171.5 ± 23.0 (6) |
| Dynorphin (15 $\mu$M) | 27.8 ± 1.9 (6)** | 250.5 ± 22.0 (6)** |
| Glucagon (1 nM) | 28.5 ± 1.5 (8)** | 247.7 ± 17.0 (8)** |
| Adrenaline (10 $\mu$M) | 24.7 ± 2.3 (8)* | 231.5 ± 16.0 (8)** |

Table 2. *Effect of naloxone on total glucose output by the cells and incorporation of [2-$^{14}$C]pyruvate into glucose*
The experimental protocol was as described in the Experimental section. Naloxone and [leucine]-enkephalin were added to final concentrations of 30 µM and 15 µM respectively. Results are means ± S.E.M. for six different cell preparations. *$P < 0.05$ and **$P < 0.01$ for significance of difference from controls.

| | Rate (nmol/h per mg wet wt.) | |
|---|---|---|
| | Glucose output | Incorporation of [2-$^{14}$C]pyruvate into glucose |
| Control | 80.6 ± 7.8 | 20.8 ± 2.5 |
| Naloxone | 80.3 ± 8.4 | 17.6 ± 2.5* |
| [leucine]Enkephalin | 221.1 ± 9.6** | 34.5 ± 3.0** |
| [leucine]Enkephalin + naloxone | 223.9 ± 10.8** | 29.5 ± 2.8** |

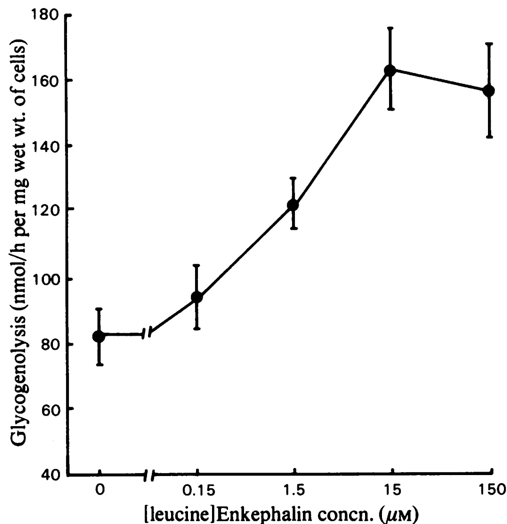


Fig. 1. *Sensitivity of the cells to [leucine]enkephalin concentration as measured by glycogen mobilization*
The experimental protocol was as described in the Experimental section. [leucine]Enkephalin was added to the final concentration indicated and the rate of glycogenolysis determined over a 15 min incubation period. Results are expressed as means ± S.E.M. for six different cell preparations.

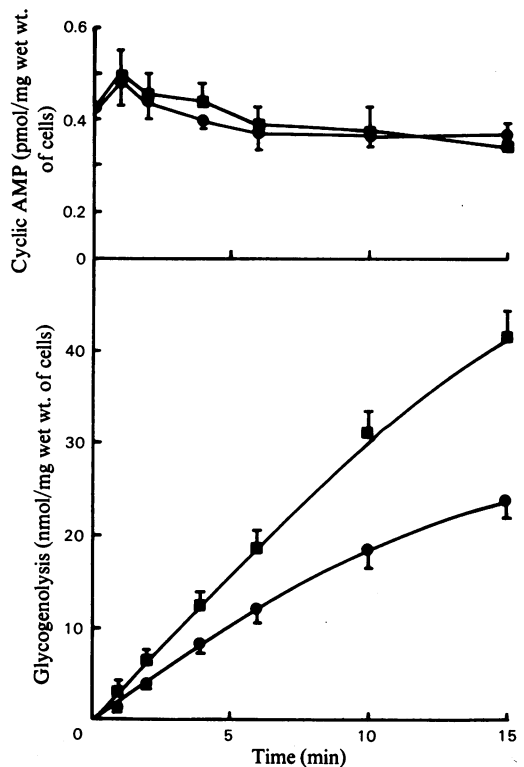


Fig. 2. *Effect of [leucine]enkephalin on the rate of glycogenolysis and cyclic AMP formation with time*
The experimental protocol was as described in the Experimental section. [leucine]Enkephalin was added to a final concentration of 15 µM at zero time, and samples were removed for the determination of cyclic AMP and glucose formation at the times indicated. Results are means ± S.E.M. for five different cell preparations. Symbols: ●, control; ■, [leucine]enkephalin.

suggests that the effects *in vivo* of morphine on gluconeogenesis may be mediated either centrally, via catecholamine release or through alterations in the plasma glucagon, insulin or vasopressin levels (Weitzman *et al.*, 1977; Ipp *et al.*, 1978; Green *et al.*, 1980), although the possibility of a long-term direct action cannot be excluded.

Fig. 1 shows the effects of a range of concentrations of [leucine]enkephalin on the rate of glycogen breakdown over a 15 min incubation period. A significant stimulation ($P < 0.01$) was apparent with all concentrations tested within the range of 1.5–150 µM. A maximal response was obtained with 15 µM-[leucine]enkephalin and a half-maximal response with 1.5 µM. An identical dose–response relation was found for gluconeogenesis (results not shown), indicating that both pathways were enhanced coincidentally. A similar sensitivity has also been demonstrated in rat vas deferens, where the half-maximal concentration of [leucine]enkephalin required to elicit a physiological response is also in the micromolar region (Miranda *et al.*, 1979; Huidobro-Toro & Leong Way, 1981). However, other tissues have been demonstrated to show greater sensitivity to opioid peptides (Lord *et al.*, 1976, 1977; Green *et al.*, 1980).

The time course for the increased rate of

glycogenolysis after addition of 15 μM-[leucine]enkephalin is shown in Fig. 2. The time of onset of the response was very rapid, being maximal within 1 min after the addition of the peptide to the cells. The difference in rate of glycogen breakdown was maintained over the entire 15 min incubation period. Fig. 2 also shows the levels of cyclic AMP in the cells plus medium at different times during the incubation period. At no time was the concentration of cyclic AMP in the treated cells elevated above that in the controls, indicating that the opioid peptides were acting via a cyclic AMP-independent mechanism to stimulate both glycogenolysis and gluconeogenesis.

Although there have been no studies documenting the existence of opiate receptors in the liver, substances with opiate-agonist-like characteristics have been shown to occur in hepatic extracts by their ability to displace bound [$^{3}$H]dihydromorphine and to inhibit the binding of [$^{3}$H]naloxone in membranes prepared from brain (Wajda *et al.*, 1976). Similarly, enzymes with the capability of degrading enkephalins have been reported to occur in liver (Shaw *et al.*, 1982), and this may suggest the existence of opiate receptors, since a parallel distribution of opiate receptors and enkephalinase activity has been demonstrated in other tissues, for example, brain (Malfroy *et al.*, 1979).

#### References

Bergmeyer, H. U. & Bernt, E. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 3, pp. 1212–1214, Academic Press, London and New York

Borrison, H. L., Fisburn, B. R., Bhide, N. K. & McCarthy, L. H. (1962) *J. Pharmacol. Exp. Ther.* **138**, 229–235

Chisholm, A. B., Allan, E. H. & Titheradge, M. A. (1983) *Biochem. J.* **214**, 451–458

Dey, P. K. & Feldberg, W. (1975) *Br. J. Pharmacol.* **54**, 163–170

Feldberg, W. & Gupta, K. P. (1974) *J. Physiol. (London)* **238**, 497–502

Green, I. C., Perrin, D., Pedley, K. C., Leslie, R. D. G. & Pyke, D. A. (1980) *Diabetologia* **19**, 158–161

Huidobro-Toro, J. P. & Leong Way, E. (1981) *Life Sci.* **28**, 1331–1336

Ipp, E., Dobbs, R. & Unger, R. H. (1978) *Nature (London)* **276**, 190–191

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. (1976) in *Opiates and Endogenous Opioid Peptides* (Kosterlitz, H. W., ed.), pp. 275–280, Elsevier/North-Holland Biomedical Press, Amsterdam and New York

Lord J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. (1977) *Nature (London)* **267**, 495–499

Malfroy, B., Swerts, J. P., Guyon, A., Roques, B. P. & Schwartz, J. C. (1979) *Neurosci. Lett.* **11**, 329–334

Miranda, HJ., Huidobro, F. & Huidobro-Toro, J. P. (1979) *Life Sci.* **24**, 1511–1518

Reid, R. L. & Yen, S. S. C. (1981) *J. Clin. Endocrinol. Metab.* **52**, 592–594

Shaw, J. S., Miller, L., Turnbull, M. J., Gormley, J. J. & Morley, J. S. (1982) *Life Sci.* **31**, 1259–1269

Wajda, I. J., Neidle, A., Ehrenpreis, S. & Manigault, I. (1976) in *Opiates and Endogenous Opioid Peptides* (Kosterlitz, H. W., ed.), pp. 129–136, Elsevier/North-Holland Biomedical Press, Amsterdam and New York

Weitzman, R. E., Fisher, D. A., Minick, S., Ling, N. & Guilleman, R. (1977) *Endocrinology* **101**, 643–645

Werther, G., Joffe, S. & Sperling, M. A. (1981) *Diabetologia* **21**, 342

Wong, S. C. & Yeung, D. C. Y. (1981) *Int. J. Biochem.* **13**, 323–327

Wong, S. C., Yeung, Y. G. & Yeung, D. (1978) *Biochem. Pharmacol.* **27**, 1347–1351