soluble sites from striatum, whereas both drugs were very active in the frontal cortex[6]. Third, a tetraline derivative, known to be the most specific dopamine agonist[6,25], and thus being inactive in the spiperone binding assay in the frontal cortex, inhibited the spiperone binding of the solubilised preparation of dog striatum at very low concentrations. Finally, the good correlation between the inhibitory effects of drugs in the soluble preparation and their antagonism of apomorphine-induced emesis is only possible if the relative high affinity of the dopamine receptors is maintained after the solubilisation process.

Thus, the above results provide evidence that the spiperone binding sites solubilised by digitonin treatment from dog striatum retain the characteristics of dopamine receptors.

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Table 1 Intrinsic molar potencies of LPH peptide fragments calculated relative to α-MSH

LPH Structure	Potency
(α-MSH)	(1.000)
61–91	5.036×10^{-4} (4.813 and 5.270×10^{-4})
66–91	3.590×10^{-4} (3.406 and 3.785×10^{-4})
87–91	1.409×10^{-4} (1.377 and 1.442×10^{-4})
87(Bz1)–91	1.667×10^{-4} (1.574 and 1.765×10^{-4})
88–91	3.245×10^{-5} (3.067 and 3.434×10^{-5})
89–91	4.427×10^{-5} (4.101 and 4.780×10^{-5})
88–90	4.266×10^{-5} (4.027 and 4.520×10^{-5})
66–89	2.967×10^{-4} (2.797 and 3.148×10^{-4})
61–65, 70–89(D-Ala^{62}, Leu^{65})	2.548×10^{-4} (2.432 and 2.670×10^{-4})
61–65, 76–89(D-Ala^{62}, Leu^{65})	2.105×10^{-4} (1.992 and 2.225×10^{-4})
61–65, 81–89(D-Ala^{62}, Leu^{65})	8.588×10^{-5} (8.269 and 8.920×10^{-5})
61–65, 85–89(D-Ala^{62}, Leu^{65})	7.586×10^{-5} (7.205 and 8.199×10^{-5})
61–65, 88–89(D-Ala^{62}, Leu^{65})	5.237×10^{-5} (4.921 and 5.573×10^{-5})
61–76	2.245×10^{-5} (2.095 and 2.406×10^{-5})
61–76 (D-Ala^{62}, Leu^{65})	8.878×10^{-5} (8.375 and 9.412×10^{-5})
61–77	2.245×10^{-5} (2.095 and 2.406×10^{-5})
61–77 (D-Ala^{62}, Leu^{65})	6.140×10^{-5} (5.884 and 6.408×10^{-5})
61–65	6.890×10^{-8} (6.764 and 7.017×10^{-8})

Dose–response curves were obtained to α-MSH and the 18 peptide sequences, using the *Anolis* rate method of MSH bioassay[2]. Using analysis of variance[8], the slope of the dose–response curves of the LPH peptide fragments did not differ significantly from that of α-MSH ($P>0.05$). The 95% fiducial limits of the estimated potencies are given in parentheses.

The *Anolis* rate method of MSH bioassay[2] was used in these experiments. The peptides Tyr-Lys-Lys-Gly-Glu (human β-LPH$_{87-91}$), its (tyrosyl) *O*-benzyl derivative [87(Bzl)-91], Lys-Lys-Gly-Glu (89–91), Lys-Gly-Glu (88–91), and Lys-Lys-Gly (88–90) were prepared by classical solution methods and characterised by TLC, paper electrophoresis and amino acid analysis of acid and/or enzymatic digests. Other peptides used in

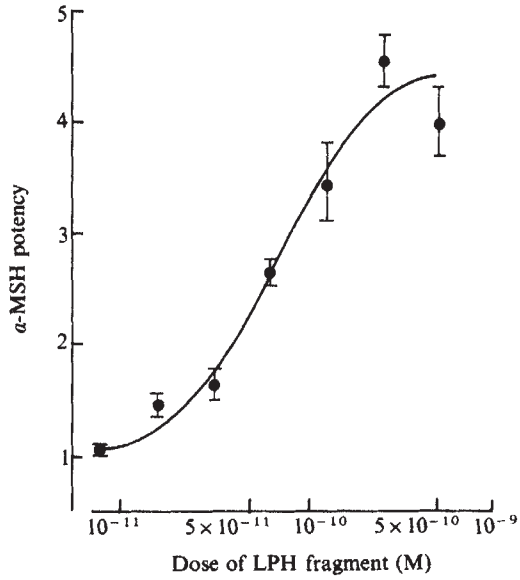

Fig. 1 The dose-related potentiation of α-MSH potency by β-endorphin. Dose–response curves were obtained from eight two-fold dilution series of α-MSH, seven of which had had added β-endorphin concentrations. The potencies of each α-MSH dose–response curve in the presence of β-endorphin concentration was calculated relative to that in the absence of added β-endorphin. Thus each point on the graph represents the increase in α-MSH potency with a β-endorphin concentration. The 95% fiducial limits of the estimated potencies are represented as vertical bars. There was a significant increase in α-MSH potency with concentrations of 16×10^{-12} M β-endorphin or greater ($P<0.01$).

Melanotropin potentiating factor is the C-terminal tetrapeptide of human β-lipotropin

WE recently demonstrated that the molar pigmentary potency of human β-lipotropin (β-LPH) is greater than other melanocyte-stimulating hormones (MSHs) on *Anolis* skin; it is 2.6–4.0 times greater than α-MSH[1] using the *Anolis* rate method of MSH bioassay[2]. We also found (unpublished) human β-LPH to be 2 to 4 times more potent than α-MSH using the steady state (quantal[3]) method. This was due to a potentiation of the MSH sequence of β-LPH (LPH$_{47-53}$) by a factor associated with β-endorphin (LPH$_{61-91}$). We showed that the melanotropin potentiating factor (MPF) was not the opiate peptide, ^{5}Met-enkephalin (LPH$_{61-65}$)[4], and now report the identification of MPF as the sequence LPH$_{88-91}$.

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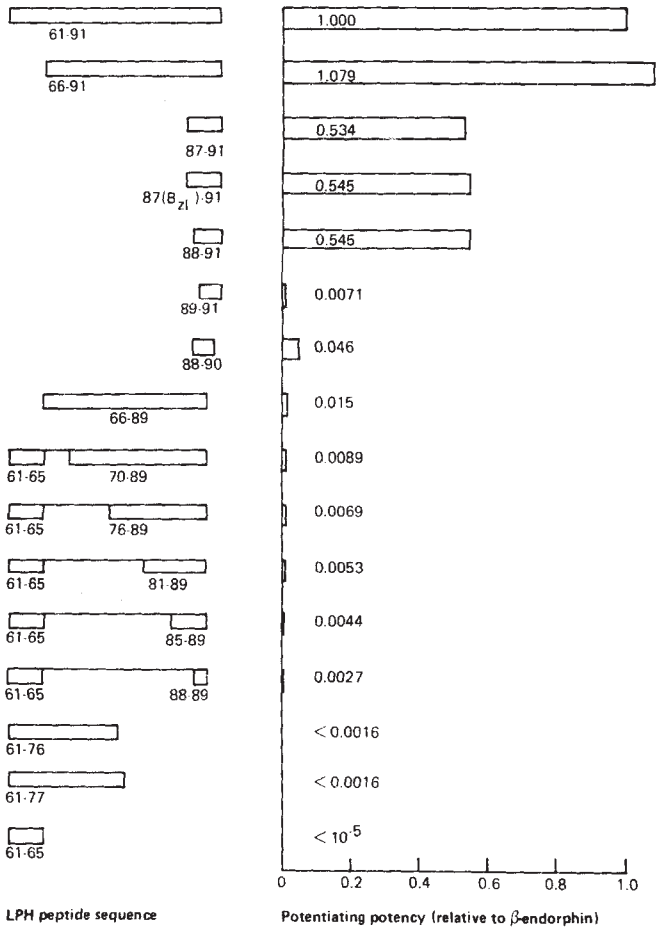

Fig. 2 Potentiating activities of LPH fragments on α-MSH potency. Bioassays of α-MSH were performed with and without LPH peptide fragments in various concentrations. The dose-related potentiation of α-MSH potency by each LPH fragment was measured as described in Fig. 1 and calculated relative to that of β-endorphin (LPH_{61-91}) using a two-factorial assay system.

these experiments were prepared by N. N. Petter (ICI) by solid phase methods[5].

Dose–response curves were obtained for α-MSH and the synthetic LPH peptide fragments and their intrinsic molar potencies were calculated (Table 1). All showed negligible intrinsic MSH activity. Constant concentrations of the LPH peptide sequences were then incorporated into the twofold dilutions used to obtain the α-MSH dose–response and their relative potencies were calculated. β-Endorphin potentiated α-MSH potency with the dose–response curves remaining parallel. This potentiation was dose-related (Fig. 1) and the α-MSH potency was increased to a maximum of 4.5-fold. To determine the sequence responsible for this potentiation, the potentiation activity of each peptide sequence was compared with that of β-endorphin (Fig. 2). Unequivocally, the results show that the sequence responsible for the potentiation is LPH_{88-91}, Lys-Lys-Gly-Glu. Thus potentiating activity was abolished by the removal of the 88th or 91st amino acids from the tetrapeptide, while extension of the sequence to include tyrosine at position 87 did not increase potentiating activity further. The higher potentiating activity of LPH_{61-91} (β-endorphin) and LPH_{66-91} than LPH_{88-91} was probably due to increased stability of the longer peptides as there was negligible activity in the sequences between LPH_{61} and LPH_{89}. These findings cannot be attributed to characteristics of the rate assay as we have obtained similar results (unpublished) with a steady state (quantal[3]) method. We therefore conclude that the melanotropic potentiating factor (MPF) is human LPH_{88-91}, Lys-Lys-Gly-Glu. Whether MPF acts by stimulation of an independent receptor or by causing conformational changes is uncertain.

As a 16×10^{-12} M concentration of β-endorphin will potentiate MSH activity significantly (see legend to Fig. 1), the effect may be physiological. Although we have tested MPF activity on the *Anolis* skin, the unexpectedly high sebotropic[6] and neurotropic[7] potencies of β-LPH in the rat suggest that MPF may also modulate MSH peptide actions in the mammal and we are presently investigating this possibility.

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Inhibition of DNA synthesis *in vitro* by binding of benzo(*a*)pyrene metabolite diol-epoxide I to DNA

IT has been shown that benzo(*a*)pyrene (BP) is a strong mutagen and carcinogen after metabolic activation by mixed function oxidases and epoxide hydratase. Evidence now indicates that (±)*r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene (diol-epoxide I) is an ultimate carcinogenic and mutagenic form of BP[1–4]. This metabolic intermediate interacts covalently with nucleic acids[5–8]. Most binding to DNA, 80% of the total, occurs by coupling between the 2-amino group of guanine and the carbon 10 position of diol-epoxide I (ref. 5). Binding to adenine, less than 15% of the total, leads to partial denaturation of the DNA double helix[6], and binding of diol-epoxide I to the phosphate groups of DNA results in DNA strand scission[8]. Intercalation of diol-epoxide I may cause conformational change of the DNA double helix[6,7]. Despite these extensive studies, the mechanism of alteration of the genetic function of DNA due to the binding of BP is not understood. We describe here a system which has enabled us to analyse the effect of BP binding to DNA on the replication of double-stranded circular DNA *in vitro*. pBR322 DNA (molecular weight, 2.6×10^6) is an artificial plasmid DNA derived from ColE1 and pBR313 in *Escherichia coli*[9]. It carries the base sequence derived from ColE1 DNA at the site of origin of replication[9]. Like ColE1 DNA[11–13], pBR322 DNA replicates semiconservatively and completely in a crude lysate of *E. coli*.

The binding of diol-epoxide I to pBR322 DNA was performed as described previously[6]. The number of diol-epoxide I molecules covalently bound to DNA, the molar ratio (MR), was determined using the radioactivity of ^{14}C-diol-epoxide I (29.4 mCi mmol^{-1}, NCI), UV absorption (254 nm), fluorescence and the molecular weight of pBR322 DNA. The covalent binding increased linearly as a function of increasing dose of diol-epoxide I concentration in the reaction mixture. High pressure liquid chromatography (Waters Bondapak C18

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