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Different effects of di-isopropylfluorophosphate on the entry of opioids into mouse brain

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1 Di-isopropylfluorophosphate (DFP) potentiates the antinociceptive activity of alfentanil but has no effect on the activity of morphine or fentanyl. We have studied the effect of DFP on the distribution of these three opioids in the brain.

2 Distribution studies were carried out using ^{3}H-labelled opioids administered subcutaneously to mice. Animals were killed at times of peak antinociceptive activity and ^{3}H-opioid measured in plasma and in eight brain regions.

3 DFP pretreatment (1 mg kg^{-1}) caused a significant increase in the brain:plasma ratio of alfentanil in all brain regions but had no effect on brain:plasma ratios for morphine or fentanyl.

4 The enhanced entry of alfentanil into the brain of DFP-treated mice probably accounts for the increased antinociception observed with this opioid. This drug interaction appears to be opioid specific.

Introduction

The irreversible anticholinesterase di-isopropylfluorophosphate (DFP) has been shown by some workers to be antinociceptive in rats, using the hot plate and tail flick tests (Koehn & Karczmar, 1978; Koehn *et al.*, 1980). In the mouse hot-plate test this effect occurs only at doses which produce marked motor impairment (Kitchen & Green, 1983). We have also shown that sub-antinociceptive doses of DFP potentiate alfentanil antinociception but have no effect on the activity of morphine or fentanyl (Kitchen & Green, 1983). Since all three opioids are μ-selective agonists it seemed unlikely that the observed interaction between DFP and alfentanil was manifested at opioid receptors; therefore, the possibility of a pharmacokinetic interaction was envisaged. The results of the present study demonstrate that DFP can increase the amount of radiolabelled alfentanil reaching the brain but has no effect on the entry of morphine or fentanyl.

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Methods

Radiolabelled distribution studies

Male albino mice (CD-1 strain, 25–30g) were equilibrated for 24 h in a quiet laboratory before experimentation. All experiments were performed between 09 h 30 min and 12 h 30 min. DFP (1 mg kg^{-1}) or 0.9% w/v NaCl solution (saline) was injected subcutaneously in a volume of 0.1 ml. This was followed 55, 50 or 30 min later by a subcutaneous injection of radiolabelled alfentanil, fentanyl or morphine respectively. Two doses of each opioid were studied and each injection contained 7 μCi of ^{3}H-labelled opioid. Animals were killed by decapitation at times of peak antinociceptive activity, as determined in previous studies (Kitchen & Green, 1983); 5 min for alfentanil, 10 min for fentanyl and 30 min for morphine. Trunk blood was collected into heparinised tubes and the brains rapidly removed. Brains were dissected over ice into eight regions as described by Glowinski & Iversen (1966) and weighed. Brain tissue was solubilized in 1 ml of Soluene-100 and 20 μl of plasma solubilized in 0.5 ml Soluene-100. Scintillation fluid (Wood *et al.*, 1975) was added to samples and ^{3}H measured in a LKB 1210 Ultrabeta scintillation counter using appropriate quench correction curves.

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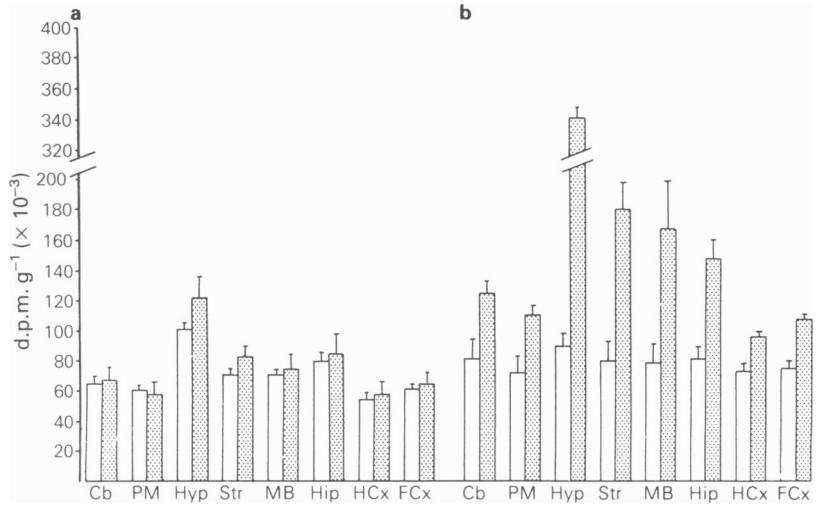

Figure 1 Effect of di-isopropylfluorophosphate (DFP) on brain levels (d.p.m. g^{-1}) of [^{3}H]-morphine (a) 10 mg kg^{-1} and (b) 20 mg kg^{-1}. Open columns: control-treated. Stippled columns: DFP (1 mg kg^{-1})-treated. Each column represents the mean and the vertical lines s.e.mean of at least six observations. Cb = cerebellum, PM = pons and medulla, Hyp = hypothalamus, Str = striatum, MB = mid brain, Hip = hippocampus, HCx = hind cortex, FCx = frontal cortex.

Drugs

Di-isopropylfluorophosphate was purchased from Sigma and morphine sulphate from MacFarlan Smith. Radiolabelled morphine was purchased from Amersham and radiolabelled fentanyl from IRE U.K. Ltd (High Wycombe). Fentanyl citrate, alfentanil hydrochloride and radiolabelled alfentanil were gifts from Janssen Pharmaceuticals.

Results

DFP treatment (1 mg kg^{-1}) caused a significant increase in brain levels of alfentanil (expressed as d.p.m. g^{-1}) in all brain regions at the 400 μg kg^{-1} dose level (Figure 3). At the lower dose of alfentanil (200 μg kg^{-1}), although brain levels were unaltered by DFP, there was a marked reduction in the plasma concentration of radiolabelled alfentanil (Table 1).

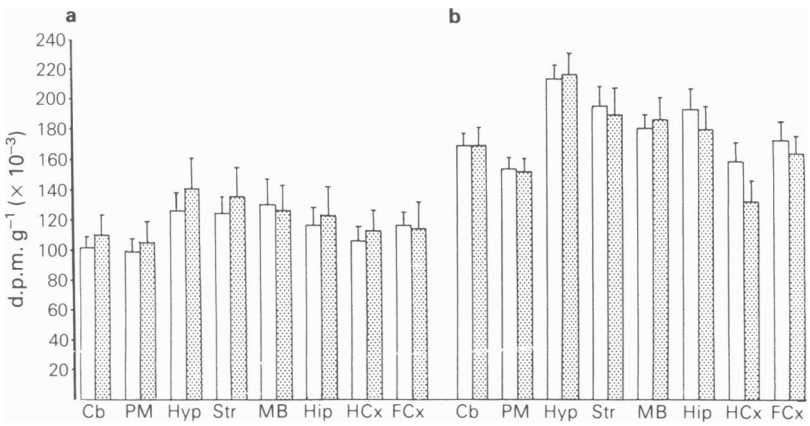

Figure 2 Effect of di-isopropylfluorophosphate (DFP) on brain levels (d.p.m. g^{-1}) of [^{3}H]-fentanyl (a) 40 μg kg^{-1} and (b) 80 μg kg^{-1}. Open columns: control-treated. Stippled columns: DFP (1 mg kg^{-1})-treated. Each column represents the mean and vertical lines s.e.mean of at least six observations. Cb = cerebellum, PM = pons and medulla, Hyp = hypothalamus, Str = striatum, MB = mid brain, Hip = hippocampus, HCx = hind cortex, FCx = frontal cortex.

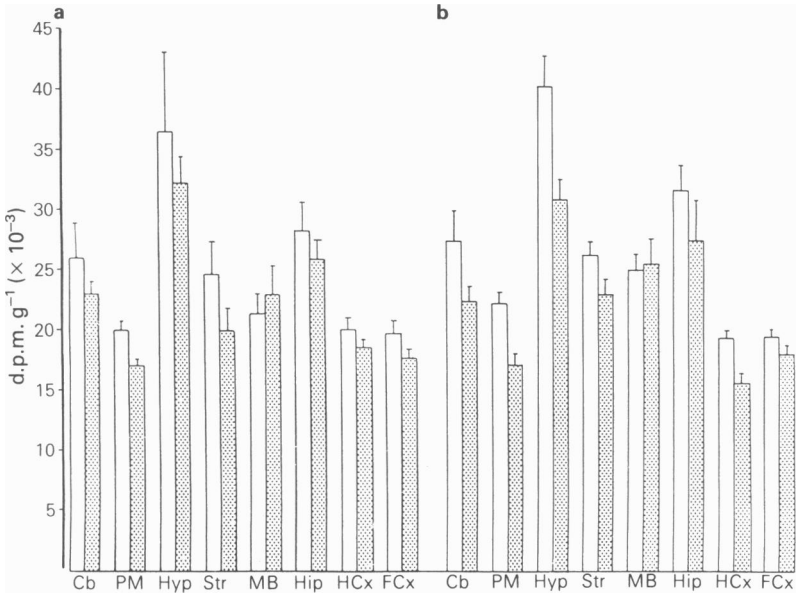

Figure 3 Effect of di-isopropylfluorophosphate (DFP) on brain levels (d.p.m. g^{-1}) of [^{3}H]-alfentanil (a) 200 $\mu g\,kg^{-1}$ and (b) 400 $\mu g\,kg^{-1}$. Open columns: control-treated. Stippled columns: DFP (1 $mg\,kg^{-1}$)-treated. Each column represents the mean and vertical lines s.e.mean of at least six observations. Cb = cerebellum, PM = pons and medulla, Hyp = hypothalamus, Str = striatum, MB = mid brain, Hip = hippocampus, HCx = hind cortex, FCx = frontal cortex.

Brain:plasma ratios of both doses of alfentanil were accordingly significantly increased by DFP in all brain regions (Table 2). The magnitude of the increase varied from 1.7 fold in the hind cortex to 3.2 fold in the hypothalamus.

DFP pretreatment had little effect on levels of morphine or fentanyl in the brain (Figures 1 and 2), and brain:plasma ratios for these two opioids were very similar in control- and DFP-treated animals (Table 2).

Table 1 Effect of di-isopropylfluorophosphate (DFP, 1 $mg\,kg^{-1}$) on plasma levels of [^{3}H]-morphine, fentanyl and alfentanil

	Plasma radioactivity (d.p.m. $ml^{-1} \times 10^{-3}$)	
Opioid	*Control*	*DFP-treated*
Morphine (10 $mg\,kg^{-1}$)	197 ± 7	174 ± 6*
Morphine (20 $mg\,kg^{-1}$)	118 ± 10	97 ± 8
Fentanyl (40 $\mu g\,kg^{-1}$)	73 ± 7	90 ± 13
Fentanyl (80 $\mu g\,kg^{-1}$)	67 ± 4	57 ± 3
Alfentanil (200 $\mu g\,kg^{-1}$)	166 ± 10	110 ± 12*
Alfentanil (400 $\mu g\,kg^{-1}$)	147 ± 34	97 ± 6

Each value is the mean of six observations.
* $P < 0.05$, DFP vs control (Student's t test).

Discussion

In control-treated animals the levels of radioactivity in the brain compared to the plasma are highest for fentanyl, followed by alfentanil and then morphine. This accords with the lipid/water partition coefficients of these opioids; fentanyl > alfentanil > morphine (Herz & Teschemacher, 1971; Stanski & Hug, 1982) a factor which markedly influences penetration through the blood-brain barrier (Brodie *et al.*, 1960). The times for measurement of morphine, fentanyl and alfentanil levels were chosen to correspond to peak antinociceptive activity determined in our laboratory. These times agree with the literature values for these opioids (Gardocki & Yelnosky, 1963; Johannesson & Becker, 1973). There is also a good correlation between levels of these opioids in the brain and their antinociceptive effects (Johannesson & Becker, 1973; Hug & Murphy, 1981).

It is clear that the effects of DFP on the distribution of radiolabelled morphine, fentanyl and alfentanil are dissimilar. The most marked effect of DFP is on the distribution of alfentanil where plasma levels are lower and at 400 $\mu g\,kg^{-1}$ alfentanil there is a concomitant rise in alfentanil in the brain. This enhanced entry of alfentanil into the brain of DFP-treated mice may account for the increased antinociception observed with this opioid (Kitchen & Green, 1983). The lack of a

Table 2 Effect of di-isopropylfluorophosphate (DFP, 1 mg kg^{-1}) on brain:plasma ratios for [^{3}H]-morphine, fentanyl and alfentanil

	Brain:plamsa ratios											
Brain region	*Morphine 10 mg kg^{-1}*		*Morphine 20 mg kg^{-1}*		*Fentanyl 40μg kg^{-1}*		*Fentanyl 80μg kg^{-1}*		*Alfentanil 200μg kg^{-1}*		*Alfentanil 400 μg kg^{-1}*	
		+DFP		+DFP		+DFP		+DFP		+DFP		+DFP
Cerebellum	0.13	0.13	0.25	0.24	1.4	1.2	2.5	2.8	0.4	0.6*	0.6	1.3*
Pons and medulla	0.10	0.10	0.20	0.18	1.4	1.2	2.3	2.7	0.4	0.5*	0.5	1.2*
Hypothalamus	0.19	0.18	0.35	0.33	1.7	1.6	3.2	3.9	0.6	1.1*	0.8	3.5*
Striatum	0.13	0.12	0.23	0.25	1.7	1.5	3.0	3.3	0.4	0.8*	0.6	1.9*
Mid-brain	0.11	0.13	0.23	0.27	1.8	1.4	2.7	3.3	0.4	0.7*	0.6	1.7*
Hippocampus	0.15	0.15	0.29	0.30	1.6	1.4	2.9	3.2	0.5	0.8*	0.6	1.6*
Hind cortex	0.10	0.11	0.17	0.17	1.5	1.3	2.4	2.7	0.3	0.5*	0.6	1.0*
Frontal cortex	0.10	0.10	0.18	0.19	1.6	1.3*	2.7	2.9	0.4	0.6*	0.6	1.1*

Each value is the mean of six observations.
* $P < 0.05$, DFP vs control (Student's t test).

significant rise in brain levels at 200 μg kg^{-1} alfentanil may be partly due to lower plasma levels since the blood volume of the brain is not accounted for in determinations of brain radioactivity.

The mechanisms underlying the interaction between DFP and alfentanil are at present unclear. Although DFP has been shown to cause increased permeability of certain blood capillaries (von Sallmann & Dillon, 1947), it would be expected that a change in the permeability of the blood-brain barrier would alter penetration of all opioids. This does not appear to be the case. Displacement from plasma protein sites should also be considered since DFP irreversibly binds to plasma proteins and to erythrocytes (Truhaut, 1966). Both fentanyl and alfentanil have a high fractional binding to blood proteins in the rat, dog and man (Meuldermans *et al.*, 1982) though data for the mouse is lacking. Again it might be expected that if DFP causes displacement, free plasma levels of both fentanyl and alfentanil would be increased allowing greater access of these opioids into brain. However, it is perhaps noteworthy that fentanyl but not alfentanil binding is markedly influenced by blood pH; DFP may produce either acidosis or alkalosis as a result of its effects on respiration (Karczmar, 1967).

In conclusion, the distribution of alfentanil and access to the central nervous system was altered by DFP. The interaction appears to be opioid specific since the entry of either fentanyl or morphine into the brain was relatively unaffected.

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