J. Pharm. Pharmacol. 1988, 40: 292–293
Communicated November 9, 1987

Differential displacement of opioids from plasma protein binding sites by di-isopropylfluorophosphate in the mouse

PAUL G. GREEN, IAN KITCHEN, *Department of Biochemistry, Division of Pharmacology and Toxicology, University of Surrey, Guildford, Surrey, GU2 5XH, UK*

Abstract—Di-isopropylfluorophosphate (DFP) displaced the opioid drug, alfentanil, from plasma proteins in-vivo and in-vitro in mice but was without effect on the structurally related compound, fentanyl. This action probably accounts for the enhanced entry of alfentanil into the brain of DFP-treated animals and its enhanced antinociceptive activity.

The irreversible anticholinesterase agent di-isopropylfluorophosphate (DFP) potentiates the antinociceptive activity of the short-acting opioid alfentanil in mice but has no effect on the activity of the related compound fentanyl nor on the activity of morphine (Kitchen & Green 1983). Further studies in our laboratories have shown that DFP enhances the entry of alfentanil into the brain (Green & Kitchen 1985) and this may account for the observed increase in antinociception. We have entertained the possibility that this may reflect enhanced free concentrations of alfentanil in plasma due to displacement from protein binding sites since this opioid exhibits a high fractional binding to blood proteins (Meuldermans et al 1982). We report here the effects of DFP on the plasma protein binding of fentanyl and alfentanil.

Methods

Male albino mice (CD-1 strain; 25–30 g) were purchased from Charles River, maintained under controlled temperature, humidity and lighting (12 h light/dark cycle; lights on 0700 h). Animals were equilibrated for 24 h in a quiet laboratory before experimentation and experimental procedures carried out between 0900 h and 1300 h. Alfentanil, fentanyl and [^{3}H]alfentanil (23 Ci mmol^{-1}) were gifts from Janssen Pharmaceuticals. [^{3}H]Fentanyl (15–18 Ci mmol^{-1}) was purchased from IRE (UK) Ltd.

Plasma protein binding was determined in-vitro and in-vivo using an ultrafiltration micropartition system. For in-vivo studies, mice were injected with 0·9% NaCl (saline) or DFP (1 mg kg^{-1}) subcutaneously 50 min before injection of fentanyl (80 μg kg^{-1}) and 55 min before alfentanil (400 μg kg^{-1}) subcutaneously, a dosing protocol chosen to parallel previous antinociceptive studies (Kitchen & Green 1983). Each injection contained 7 μCi of [^{3}H]opioid. Trunk blood was collected following decapitation at 60 min (a time equivalent to the peak antinociceptive effects of each opioid) and plasma obtained by centrifugation (2500 *g* for 10 min). For in-vitro studies mice received saline or DFP (1 mg kg^{-1}) subcutaneously and trunk blood was collected 60 min later. 250 μL of plasma was incubated for 30 min at 30°C with 25 μL [^{3}H]alfentanil or [^{3}H]fentanyl. The incubation medium contained 17 nCi of radiolabelled opioid plus additional unlabelled drug to provide plasma concentrations paralleling those observed in-vivo.

For both in-vivo and in-vitro studies free and protein bound plasma concentrations were determined by ultrafiltration using an Amicon micropartition Kit (MPS-1). 200 μL plasma aliquots were centrifuged in partition tubes at 1500 *g* for 10 min. Tritium was determined in 20 μL aliquots of the ultrafiltrate (unbound drug) and 20 μL of original plasma (total drug) by scintillation counting. Recovery experiments were performed to determine loss of alfentanil or fentanyl during ultrafiltration due to adsorption on membranes or the plastic reservoir.

Correspondence to: I. Kitchen, Dept of Biochemistry, Division of Pharmacology & Toxicology, University of Surrey, Guildford GU2 5XH, UK.

Results

Recovery of alfentanil and fentanyl using the ultrafiltrate method was 103 ± 11 and 80·7 ± 1·3% (n = 6), respectively. Table 1 shows the effect of DFP on the total plasma and free plasma alfentanil and fentanyl. DFP pretreatment caused a decrease in plasma protein binding of alfentanil both in-vivo and in-vitro, resulting in a > 50% increase in free concentration of the drug in plasma. In-vivo, this was accompanied by a decrease in total alfentanil in the plasma indicating redistribution of free drug throughout the animal. In contrast, DFP did not alter total or free concentrations of fentanyl in both in-vivo and in-vitro experiments. However, fentanyl binding in both control and DFP-treated groups was markedly higher in-vitro than in-vivo (Table 1).

Table 1. Effect of DFP (1 mg kg^{-1}) treatment on in-vivo and in-vitro plasma levels of alfentanil and fentanyl (ng mL^{-1}).

Treatment		Alfentanil		Fentanyl	
		in-vivo	in-vitro	in-vivo	in-vitro
Saline	Total	485 ± 25	519 ± 5	13·3 ± 0·8	16·9 ± 0·4
	Free	118 ± 6	140 ± 10	7·1 ± 0·5	2·0 ± 0·1
	% Free	24·3%	26·9%	53·5%	11·9%
DFP	Total	286 ± 40	517 ± 9	12·3 ± 1·5	16·3 ± 0·6
(1 mg kg^{-1})	Free	110 ± 13	195 ± 12	5·6 ± 0·7	1·8 ± 0·1
	% Free	38·5%	37·8%	46·0%	11·2%

Values are the mean ± s.e.m. of 6 observations.

Discussion

Plasma protein binding of alfentanil has been reported at 84, 73 and 92% for rat, dog and man, respectively (Meuldermans et al 1982). The results for mice reported here fall within this range. Fentanyl binding in-vitro in the mouse (89%) is also similar to that obtained by Meuldermans et al (1982) for the rat, dog and man. In-vivo binding however shows a much lower fractional binding which has been observed by other workers in the dog (Murphy et al 1979). The difference between in-vitro and in-vivo binding data is not clear but may be related to temperature and pH differences in the protocols since fentanyl binding is highly dependent on these two parameters (Hollt & Teschemacher 1975). The in-vitro methodology employed a 30 min incubation step and as the pH of plasma rises when left in air higher fractional binding of fentanyl may be the result of this. It is unlikely to be related metabolism since measures of binding have been made at times when metabolism of these compounds is minimal (Meuldermans et al 1982).

DFP is known to bind plasma proteins (Martin 1985) and is clearly capable of displacing alfentanil from its binding sites. The resultant increase in free drug probably accounts for the enhanced entry of this opioid in to the central nervous system of

DFP-treated animals (Green & Kitchen 1985). DFP does not enhance fentanyl entry in to the brain (Green & Kitchen 1985) and the lack of effect on plasma protein binding accords with this observation. The differential interaction of these two structurally related opioids may reflect the different proteins which they bind. Alfentanil is predominantly bound in plasma to α_1-acid glycoprotein whilst fentanyl binds red blood cells, glycoproteins and lipoproteins (Meuldermans et al 1982). It seems likely therefore that DFP is displacing alfentanil from α_1-acid glycoprotein sites, though the possibility of irreversible phosphorylation of the acceptor proteins cannot be excluded.

We are grateful to Janssen Pharmaceuticals for the gift of [^{3}H]alfentanil, alfentanil and fentanyl.

References

Green, P. G., Kitchen, I. (1985) Different effects of di-isopropylfluorophosphate on the entry of opioids into mouse brain. Br. J. Pharmacol. 84: 657–661

Hollt, V., Teschemacher, H. (1975) Hydrophobic interactions responsible for unspecific binding of morphine-like drugs. Naunyn Schmeidebergs Arch. Pharmacol. 288: 163–177

Kitchen, I., Green, P. G. (1983) Differential effects of di-isopropylfluorophosphate poisoning and its treatment of opioid antinociception in the mouse. Life Sci. 33: 669–672

Martin, B. R. (1985) Biodisposition of [^{3}H]di-isopropylfluorophosphate in mice. Toxicol. Appl. Pharmacol. 124: 64–72

Meuldermans, W., Hurkmans, R. M. A., Heykants, J. J. P. (1982) Plasma protein binding and distibution of fentanyl, sufentanil, alfentanil and lofentanil in blood. Arch. Int. Pharmacodyn. 257: 4–19

Murphy, M.R., Olsen, W.A., Hug, C.C.Jr (1979) Pharmacokinetics of ^{3}H-fentanyl in the dog anesthetized with enflurane. Anesthiology 50: 13–19

J. Pharm. Pharmacol. 1988, 40: 293–295
Communicated June 8, 1987

Flavonoids, leucocyte migration and eicosanoids

N. MASCOLO, A. PINTO, F. CAPASSO, *Departament of Experimental Pharmacology, University of Naples, via L. Rodinò 22, 80138 Naples, Italy*

Abstract—Quercetin reduced the concentration of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) in the pleural exudate induced in rats by 1% carrageenan given intrapleurally. Leucocyte migration in the exudate was also reduced by the flavonoid. Inhibition of eicosanoids and leucocytes in the exudate was dose-related. Quercetin also reduced LTB_4 synthesis in cells stimulated with ionophore A23187, either ex-vivo or in-vitro. A similar, though less active, mode of action was found with quercitrin, while apigenin and luteolin reduced leucocyte accumulation and PGE_2 formation, but not LTB_4-formation.

It is now known that flavonoids, phenolic pigments widespread in the plant kingdom, can induce a wide range of biological effects, including enzyme inhibition, plant growth regulation and mutagenicity of bacterial testing strains (see Pagonis et al 1986).

Recently it has been observed in-vitro that some of these natural products are potent inhibitors of cyclic AMP phosphodiesterase (cAMP-PDE) in many human and animal tissues (Berets et al 1986). Flavonoids also inhibit platelet aggregation (Gryglewski et al 1985, 1987) and enzymes involved in arachidonic acid metabolism, i.e. cyclooxygenase and lipoxygenase enzymes, though various flavonoids differ in the action on cyclooxygenase and lipoxygenase pathways (Palmer & Salmon 1985).

We have evaluated in the rat the effects of quercetin, quercitrin, luteolin and apigenin on the concentration of PGE_2 and LTB_4 in experimentally induced inflammatory exudate and their relationship with leucocyte numbers migrating into the pleural cavity.

The activity of flavonoids was compared with that of indomethacin and compound BW 755C.

Correspondence to: F. Capasso, Dept of Experimental Pharmacology, University of Naples, via L. Rodinò 22, 80138 Naples, Italy.

Materials and methods

Male Wistar-Nossan rats (80–90 g) were used. Carrageenan pleurisy was induced as described previously (Capasso et al 1975). 0·2 mL of 1% carrageenan (Viscarin) suspension was injected intrapleurally into rats and 6 h after injection, the animals were killed using ether anaesthesia, exsanguinated from the carotid artery and exudate collected in polypropylene tubes at 0°C. A sample (10 μL) of the exudate was removed immediately for determination of the leucocyte count according to Hurley et al (1966) while the remainder was centrifuged at 2000 *g* for 5 min at 0°C to precipitate cells and debris. Enzymic activity was terminated with methanol–formic acid (1 mL 20 μL) and PGE_2 and LTB_4 extracted into $CHCl_3$ (2 mL) then evaporated to dryness under N_2. PGE_2 and LTB_4 were determined by radioimmunoassay. The specificity of these antisera and the procedure were reported by Autore et al (1987).

Quercetin (2–24 mg kg^{-1}), quercitrin (2–24 mg kg^{-1}), luteolin (2–24 mg kg^{-1}), apigenin (2–24 mg kg^{-1}), indomethacin (1–9 mg kg^{-1}) and compound BW 755C (10–50 mg kg^{-1}) were given intraperitoneally after intrapleural injection of carrageenan. The flavonoids were dissolved in ethanol and then diluted with 0·9% NaCl (saline); indomethacin (Indoxen) and BW 755C were dissolved in saline directly.

In some experiments a portion (500 μL) of the exudate, collected 6 h after carrageenan injection, was used to assess the capacity of the inflammatory cells to synthesize LTB_4 ex-vivo. The formation of LTB_4 by cells in the inflammatory exudate from drug-treated animals was assayed as follows. After 30 min pre-incubation of exudate (500 μL) at 37°C, ionophore A23187 (5 μg, 10 μL) was added and incubation continued for 5 min at 37°C. After centrifugation at 2000 *g* for 5 min, the supernatant was removed and assayed for LTB_4. In other experiments, 500 μL of pooled inflammatory exudate (collected 6 h after carrageenan injection and containing 5×10^6 cells) from control rats was incubated at 37°C for 30 min before addition of drug (1–100 μg,