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κ-Opioid diuretic effects of tifluadom, a benzodiazepine opioid agonist

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Tifluadom produced a dose-related (0·08–5 mg kg^{-1}) diuresis in normally hydrated rats. This diuretic effect was antagonized by κ-antagonist doses of naloxone and blocked by morphine administration. This is further in-vivo evidence that tifluadom is a fairly specific κ-opioid agonist.

The benzodiazepine derivative tifluadom has analgesic effects that are blocked by the opioid antagonist naloxone but not by the benzodiazepine antagonist R015-1788* (Romer et al 1982). Studies with isolated tissue preparations of guinea-pig ileum, mouse vasa deferentia, and rabbit vasa deferentia suggested that tifluadom had a preferential agonist activity at κ-opioid receptors. The fact that MR-2266* was more potent than naloxone in antagonizing the analgesic effects of tifluadom and the other κ-agonists bremazocine and ketazocine, whereas naloxone was more potent than MR-2266 in antagonizing morphine, also suggested that tifluadom was a κ-opioid agonist (Romer et al 1982).

κ-Opioid agonists have recently been shown to produce a marked diuretic effect in normally hydrated rats (Leander 1983a, b). This effect is apparently due to suppression of plasma vasopressin levels. At appropriate doses, the κ-agonists ketazocine, ethylketazocine, bremazocine, proxorphan and U-50,488H* all produce urinary outputs of greater than 14 ml within 5 h compared with 2 to 4 ml in control-treated rats (Leander 1983a, b). In contrast, the μ-agonists morphine and (−)-methadone only exhibit antidiuretic effects. The μ-opioid antidiuretic effects appear to be due to suppression of bladder emptying (Roppolo et al 1983). The partial κ-agonists nalorphine, butorphanol and oxilorphan increase urine output to a lesser maximum than full κ-agonists and also antagonize the maximal output produced by 0·08 mg kg^{-1} of bremazocine (Leander 1983c). The specificity of this diuretic effect as κ-receptor-mediated was also demonstrated by the fact that MR-2266 was more potent than naloxone in antagonizing the effects of bremazocine and U-50,488H (Leander 1983a, b).

The present communication reports that tifluadom produced a diuretic effect similar to other κ-opioid agonists. This effect was antagonized by naloxone and could be suppressed by morphine. The specific methods used were as previously described (Leander, 1983a, b).

* R015-1788 is ethyl 8-fluoro-5,6-dihydro-5-methyl)-6-oxo-4H-imidazol[1,5-a][1,4]-benzodiazepine 3-carboxylate MR-2266 is (−)5,9α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan HCl. U-50,488H is *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidynyl)cyclohexyl]benzene acetamine methane sulfonate.

Long-Evans hooded rats (hydrated), ca 400 g, were injected s.c. with various doses of drug and then placed in metabolism cages. Excreted urine was funnelled into graduated cylinders, and the volume was recorded at 2 and 5 h after injection. The rats had free access to food and water when not in the metabolism cages. Tifluadom was dissolved in distilled water with the aid of HCl, whereas naloxone HCl and morphine sulphate were dissolved in distilled water only. Doses are calculated as the salt for naloxone and morphine and as the base for tifluadom. When two injections were required, they were administered on opposite sides of the body in volumes of 1 ml kg^{-1}.

Results

Tifluadom markedly increased urine output, with the dose of 5 mg kg^{-1} producing a 5-h urine volume similar to the maximal diuretic effect produced by other κ-agonists (Table 1) (Leander 1983a, b). The diuretic effect produced by 0·64 mg kg^{-1} of tifluadom was antagonized by naloxone in a dose-related fashion, and could be suppressed by 20 mg kg^{-1} of morphine. As with other κ-agonists, doses of naloxone necessary to antagonize the diuretic effects were much higher (~10x) than those necessary to antagonize the effects of μ-agonists.

It is interesting that morphine suppressed markedly the diuretic effect of tifluadom as reported previously for other κ-agonists such as ketazocine and U-50488H (Leander 1983b). However, the diuretic effects of two other κ-agonists, proxorphan and bremazocine, are not

Table 1. Urinary output in rats after injection of various doses of tifluadom alone (above) and simultaneously with naloxone or morphine (below). Data are the cumulative mean (± s.e.m.) output in ml at 2 and 5 h after injection.

		Tifluadom alone	
Dose mg kg^{-1}	n	2 h	5 h
0 (control)	4	1·1 ± 0·5	2·4 ± 0·2
0·08	5	2·6 ± 0·8	3·9 ± 0·9
0·32	4	9·6 ± 1·0	11·5 ± 1·2
1·25	5	9·3 ± 1·9	11·2 ± 1·7
5·0	5	11·1 ± 2·6	18·7 ± 1·4
		0·64 mg kg^{-1} Tifluadom plus naloxone or morphine	
+0 (control)	5	9·8 ± 1·5	10·8 ± 1·8
+1 naloxone	5	5·6 ± 0·8	6·8 ± 0·6
+10 naloxone	5	2·7 ± 0·5	5·5 ± 0·8
+20 morphine	5	0·4 ± 0·2	4·4 ± 1·1

affected markedly by morphine (Leander 1983b). This may be explained by the fact that proxorphan and bremazocine, but not ketazocine or U-50488H,* have μ-opioid receptor antagonist activity. These results may indicate that tifluadom also lacks antagonist activity at μ-receptors.

The present results further support the in-vitro observation that tifluadom is a fairly specific κ-opioid agonist without actions at the μ-opioid receptor. Tifluadom should be a useful tool in the further study of κ-receptor functions.

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Fluosol 43 intravascular persistence in mice measured by ^{19}F nmr

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Quantitative determination of the intravascular persistence of F-tri-n-butylamine (FC 43 as Fluosol 43) in mice was carried out using ^{19}F nmr spectroscopy. The method allows the direct study of whole blood, neither separation nor extraction of the sample being required. Accuracy (reproducibility) is better than ±3%, and is comparable to that of the gas chromatographic (gc) method. The sensitivity of detection is less than that of the gc method but is sufficient for this biological study. It was observed that the intravascular elimination of F-tri-n-butylamine follows a non-linear kinetic and becomes faster about 40 h after the injection. This phenomenon may be explained by the size-increase of the FC 43 droplets in the emulsion. Indeed, at about 40 h after injection, the level of Pluronic F-68 in the bloodstream was no longer sufficient to maintain the stability of the FC-43 droplets. They therefore tended to coalesce forming larger droplets that were phagocytosed more rapidly by the histiocytes of the reticuloendothelial system.

Highly fluorinated organic compounds are excellent solvents for oxygen and carbon dioxide and they can be emulsified to form particles small enough to circulate in the micro blood vessels. Hence, perfluorochemical particles can effect the main function of erythrocytes. The other functions of blood, such as transport of ions and nutrients and holding osmotic and oncotic pressures, are maintained by the aqueous phase which is formulated to simulate the plasma (Clark & Gollan 1966; Sloviter & Kamimoto 1967; Geyer et al 1968; Le Blanc & Riess 1982).

Perfluorochemical emulsions must be non-toxic and should meet the two following requirements: (i) they must remain in the circulating blood for a reasonable time (i.e. they must offer good in-vivo stability), (ii) they must be rapidly eliminated from the body to avoid side effects.

It is therefore necessary to know the residence time in the bloodstream and the distribution throughout the body of perfluorochemicals and also whether long-term retention of these compounds occurs in various organs.

The amounts of perfluorocompounds in body fluids and organs have been determined by specific gravity (Clark et al 1974), by sodium biphenyl reagent combustion followed by determination of the fluoride ion released (Clark et al 1974; Stein et al 1975) or by gas chromatography (gc) after extraction with various solvents (Yamanouchi et al 1975; Cao et al 1981).

These methods lack simplicity and/or accuracy: the measurement of specific gravity only indicates the overall effect; the recovery of fluoride ion after sodium biphenyl combustion is not complete; the gc method needs extraction which is time-consuming and limits its accuracy: when a known amount of perfluorochemical in emulsified form is added to blood, the recovery is over 94% with a standard deviation (s.d.) of ±3·5% (Yamanouchi et al 1975) or over 91% with s.d. ±5% (Cao et al 1981).

We now report a new and direct assay method for the determination of perfluorochemicals in whole blood using fluorine-19 nmr (^{19}F nmr), with which we have investigated the intravascular persistence of Fluosol 43 in mice.

Fluosol 43 is one of the three commercial emulsions manufactured by the Green Cross Corporation (the other two are Fluosol-DA 20% and 35% which are mixtures of two perfluorochemicals (F-tri-n-propyl-

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