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Brain Passage of BUBU, a Highly Selective and Potent Agonist for δ Opioid Receptors: In Vivo Binding and μ Versus δ Receptors Occupancy

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DELAY-GOYET, P., M. RUIZ-GAYO, A. BAAMONDE, G. GACEL, J.-L. MORGAT AND B. P. ROQUES. *Brain passage of BUBU, a highly selective and potent agonist for δ opioid receptors: In vivo binding and μ versus δ receptors occupancy.* PHARMACOL BIOCHEM BEHAV **38**(1) 155–162, 1991.—The peptidase-resistance and bioavailability of BUBU [H-Tyr-D.Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu)-OH], a highly selective and potent agonist of the δ opioid receptor, have been investigated in vitro and in vivo. In vitro at 37°C, the peptide was fully resistant to degradation by rat serum and strongly resistant to degradation by rat brain membranes. In vivo 0.065% of the dose of [^{3}H]BUBU injected intravenously to the mouse was present 15 min later in the brain. The percentage determined for [^{3}H]DAGO [H-Tyr-D.Ala-Gly-(NMe)Phe-Gly-ol], a selective ligand for μ sites, was 0.038%. Specific binding to mouse brain membranes, determined after intracerebroventricular injection of [^{3}H]BUBU, was saturable and a high affinity (K_{Dapp} = 25 pmol) was evaluated for the δ-agonist. Competition experiments showed that BUBU is a selective ligand for δ receptors in vivo. Comparison of the analgesic potency (hot plate test) of ICV or IV administered increasing doses of BUBU and DAGO with their in vivo binding properties supports the preferential involvement of μ receptors in supraspinal analgesia. BUBU also induced an increase in spontaneous locomotion after IV administration at a dose lower than that which produced analgesia. The quantitative results obtained in the present study demonstrate that BUBU and DAGO could be used to characterize the pharmacological responses induced by selective stimulation of δ and μ receptors after systemic administration.

Analgesia Blood-brain barrier Delta opioid agonist Enkephalins DAGO BUBU Behaviour Locomotion Mu opioid agonist Opioid receptor Intravenous administration Tritiated probe In vivo binding Receptor occupancy

THE central effects obtained after peripheral administration of neuropeptides or analogues suggest a passage through the blood-brain barrier (BBB) for these molecules (8, 28, 29, 37, 48). This is the case for some opioid peptides that can elicit analgesia or behavioural effects after IV administration (5, 36, 39, 47). However, these effects could be due to the stimulation of peripheral receptors (18, 22, 41, 43) or to an opioid-induced modulation of the BBB permeabilities of other compounds (2). In addition, peptides have very short half-life due to a rapid degradation by peptidases (3,35) and are hydrophilic, making their passage through the BBB difficult. These problems can be circumvented by protecting the amide bonds and introducing hydrophobic amino acids or nonpolar protective groups.

In the field of opioid peptides, this approach has yielded selective μ analogues systemically active (5,39). Peptides selective for the δ type receptor like DSLET and DTLET were also found to be active after IV administration (4, 33, 34, 44). However, the use of DTLET as δ ligand in vivo is hindered by its residual cross-reactivity with μ sites, its relatively rapid degradation (31) and its hydrophilic nature, which does not favour its uptake at the blood tissue interface of the blood cerebrospinal fluid barrier.

In this paper we report the in vivo binding properties and passage across the BBB of BUBU [H-Tyr-D.Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu)-OH], a new lipophilic derivative of DSLET (19). In vitro binding experiments (12) have shown that BUBU is a molecule with an affinity for the δ sites close to that of DSLET and DTLET but with a greater selectivity ($K_I\mu/K_I\delta = 280$). The in vitro stability of BUBU was tested against serum and rat brain

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membranes, showing its resistance to enzymatic degradation. In vivo stability assays, binding properties and biodisponibility after peripheral administration of BUBU have been compared to those obtained using [^{3}H]DAGO, a selective ligand for μ binding sites (21). The behavioural and analgesic responses after IV injection of BUBU and DAGO were also compared and the controversial issue (26) of the major role of μ and not δ opioid receptors in supraspinal analgesia (7, 10, 16) is discussed in light of the μ and δ receptor occupancy by BUBU and DAGO, determined after IV or ICV administration at doses corresponding to their ED_{50} values.

METHOD

Animals

Male Swiss Albino mice weighing 18–20 g and male Sprague-Dawley rats weighing 140–180 g were purchased from Depré (France). Animals were housed in groups of twenty (mice) or ten (rats) with food and water made available ad lib.

Materials

BUBU [H-Tyr-D.Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu)-OH] was synthetized in the laboratory as previously described (12). Met-enkephalin was from Bachem A.G. [3,5-^{3}H]BUBU (2.77 TBq/mmol) and [^{3}H]DSTBULET {[3,5-^{3}H]Tyr-D.Ser(OtBu)-Gly-Phe-Leu-Thr-OH, 0.87 TB/mmol}, were prepared from their dibromo precursors (17). [^{3}H]DAGO, {[3,5-^{3}H]Tyr-D.Ala-Gly-(NMe)Phe-Gly-ol, 1.85 TBq/mmol} was from the C.E.A., [^{3}H] etorphine (1.85 TBq/mmol) was from Amersham.

In Vitro Study of the Hydrolysis of BUBU by Rat Serum and Rat Brain Membranes

Rat brain membranes were prepared as previously reported (12). Twenty μl of 10^{-3} M BUBU or Met-enkephalin (final concentration: 10^{-4} M) were incubated at 37°C during various times with 180 μl of rat serum (final concentration: 6 mg of protein/ml) or 180 μl of a rat brain membrane preparation (final concentration: 1.75 mg of protein/ml). The reaction was stopped by adding 300 μl ethanol. The tubes were centrifuged and the supernatant stored at −80°C until analysis. Fifty μl samples of the supernatant were injected in a Waters HPLC apparatus equipped with a C_{18} μ-bondapak column (3.9 × 300 mm) with a particle size of 5 μm. The elution was carried out with a 25 mM triethylammonium phosphate (pH 6.5) buffer/acetonitrile (63:37) mixture, at a flow rate of 1.2 ml/min. Detection was performed by UV absorption at 210 nm. No significant differences in BUBU hydrolysis were found when mouse serum was used in place of rat serum or when mouse brain membranes were used in place of rat brain membranes (data not shown).

Determination of the Rate of Passage of [^{3}H]BUBU and [^{3}H]DAGO Into Mouse Brain

The radioactive probes (pure or in isotopic dilution) were injected intravenously (IV) in the tail in a volume of 50 μl using a Hamilton syringe (705 RN) with a 26S gauge needle. Mice were sacrified 15 min later by decapitation. The brain was quickly removed on ice, heated at 95°C for 5 min in 1.5 ml water and then homogenized with 5 strokes of a teflon pestle in a Potter-Elvehjem tissue grinder. The homogenate was incubated at 95°C during 5 min and centrifuged for 35 min at 90,000 × g (these conditions of heating did not induced a degradation of [^{3}H]BUBU). The supernatant, containing 90% of the total radioactivity, was concentrated under vacuum, dissolved in 250 μl of HPLC buffer and centrifuged supernatants (50 μl) were coinjected with 50 μl of a 10^{-4} M solution of the corresponding nonlabelled peptide in an HPLC (Waters apparatus equipped with a Nucleosil C18 column 5 μm 24 × 0.6 cm) and eluted with 25 mM triethylammonium phosphate (pH 6.5) buffer, acetonitrile (linear gradient from 30 to 60% of acetonitrile in 20 min for [^{3}H]BUBU and 20 to 30% in 10 min for [^{3}H]DSTBULET, 23% acetonitrile for [^{3}H]DAGO, isocratic conditions) at a flow rate of 1.2 ml/min. Fractions of 0.6 ml were collected, mixed with 5 ml of Biofluor and radioactivity counted using a 1209 Rackbeta (LKB) counter. Ten μl of blood, collected during the decapitation, were diluted in 1 ml water and mixed with 15 ml of Biofluor to determine radioactivity. Standards prepared from the radioactive probes and brains were compared to the experimental samples to calculate the percentage of the administered dose in the brain (yields of recovery: 24% for [^{3}H]BUBU; 15% for [^{3}H]DSTBULET; 13% for [^{3}H]DAGO).

Binding of [^{3}H]BUBU to Mouse Brain In Vivo

Mice were injected with different doses of [^{3}H]BUBU (pure or in isotopic dilution) IV (volume: 50 μl) or ICV (volume: 5 μl) routes. ICV injections were carried out as previously described (20). The animals were sacrified 15 min after the injection and the brain quickly removed and homogenized for 10 s in 10 ml of ice-cold 50 mM Tris-HCl/0.1% bovine serum albumin/0.01% bacitracin (pH 7.4) buffer using a Polytron. Four aliquots of 0.15 ml were immediately filtered through Whatman GF/B glass filters, which had been soaked in the same buffer for 1 hour. The filters were washed with 2 × 5 ml ice-cold buffer and placed in a scintillation vial. Fifteen ml of Biofluor were added and the radioactivity (total bound ligand) was counted. Total radioactivity in the homogenate was determined by counting a 0.6 ml aliquot of the homogenate in 15 ml of Biofluor. The nonspecific binding was calculated from a standard plot describing the radioactivity bound to the membranes as a function of total radioactivity measured in the brain of mice who received 35,000 pmol (IV) or 3,000 pmol (ICV) of BUBU with different doses of [^{3}H]BUBU. Saturation data were obtained from Scatchard analysis. For each concentration, the specific binding was derived by subtracting from each experimentally determined total binding the calculated value for nonspecific binding. Competitions were carried out using 32 pmol of [^{3}H]DAGO or 25 pmol of [^{3}H]DSTBULET injected with or without various quantities of BUBU. Data from these competition experiments were analyzed as the ratio of bound to total radioactivity, as described before (31). No specific binding potency was found for metabolites of BUBU (data not shown). The occupancy of binding sites by a ligand was obtained by calculating the ratio $L/(L + K_{Dapp})$ where L is the dose of the injected ligand and K_{Dapp} its apparent in vivo equilibrium dissociation constant (K_{Dapp} represents the ICV drug dose which is necessary to occupy 50% of the total binding site population at the time of sacrifice). When the K_{Dapp} value of a ligand was not available, its IC_{50} value was used. For example, the occupancy of μ sites by IV injected DAGO (K_{Dapp} = 52 pmol) at its ED_{50} (ED_{50} IV = 41 pmol) was: $L/(L + K_{Dapp}) = 41/(41 + 52) = 0.441 = 44.1\%$ (Table 2).

Pharmacological Experiments

For spontaneous motor activity measures, the apparatus used consisted of a housing cage placed on the floor of an Animex Activity Meter type S (LKB, Farad, Sweden), in which an electromagnetic field is disturbed, resulting in an impulse when the

activity of the animal exceeds a preset level (24). Furthermore, rearings counted by the experimenter were recorded. After habituation for 5 min, distilled water or drugs were IV injected to preselected mice assigned with score of 30–65 rearings during the habituation period. Each animal was only used once and tested for 15 min after the injection. Dose-response curves were drawn with the cumulated values (number of counts) obtained during all this period.

Analgesic potencies were evaluated on the hot plate test at 55 ± 0.5°C. The hot plate was surrounded by a cylindrical Plexiglas chimney (16.5 cm diameter × 16 cm high). The jump latency time was measured using a stopwatch 15 min after IV injection. Mice that did not jump within 240 s were removed (cut-off time). The results were expressed as a percentage of analgesia according to the formula (Tt − Tc/240 s − Tc) × 100 (Tt and Tc are the jump latency times of treated and control animals, respectively). The ED_{50}'s and their 95% confidence limits were obtained according to the method of Litchfield and Wilcoxon (30). For these experiments both BUBU and DAGO were solubilized in distilled water. For analgesic experiments, one equivalent of L-lysine was added to the solution of BUBU (to improve its solubility). L-Lysine itself had no effect in the test (data not shown). Doses required for pharmacological assays were obtained by dilution of the stock solutions with distilled water. In all cases, the agonists were administered IV to restrained animals, in a final volume of 0.1 ml/10 g. Data were analysed by a one-way analysis of variance, followed by Dunnett's *t*-test.

RESULTS

In Vitro Study of the Hydrolysis of BUBU by Rat Serum or Rat Brain Membranes

No degradation of BUBU was observed after 2 hours incubation at 37°C with rat serum, in contrast to Met-enkephalin, which was degraded by 95% in 40 min ($t_{1/2} = 7$ min) (Fig. 1A). With rat membranes, 32% of the peptide was degraded after 2 hours at 37°C, giving an half-life $t_{1/2} = 210$ min (extrapolated from the plot) 42-fold greater than that of Met-enkephalin ($t_{1/2} = 5$ min) (Fig. 1B). The degradation study of DSTBULET using crude rat brain membranes has been already published (12).

Passage of Radiolabelled Probes Into Mouse Brain After IV Administration

The total radioactivity present in the brain 15 min after IV injection of the probe into mice was determined after subtraction of the radioactivity (counted using 10 μl of blood) accounting for cerebral blood (6) (Table 1). The quantity of the intact probe was determined by HPLC (Fig. 2) and, taking into account losses due to the extraction and concentration procedures (see the Method section), the percentage of the injected probe present in the brain was then calculated.

This was found to be 0.065% for [^{3}H]BUBU present in the mouse brain, whilst for [^{3}H]DSTBULET it was 9-fold lower (0.0075%). The percentage of [^{3}H]DAGO (0.038%) was similar to that obtained with [^{3}H]BUBU. [^{3}H]Etorphine, a highly lipophilic and very efficient opiate, had the best penetration (1.8%).

Binding of [^{3}H]BUBU to Mouse Brain After IV or ICV Administration

The specific binding of [^{3}H]BUBU in mouse brain was studied 2, 15, 30, 45 and 60 min after IV injection of 20, 60, 200 and 800 pmol. The nonspecific binding was determined by coinjection of 35,000 pmol of unlabelled BUBU. Three mice were used for each concentration, and each determination was performed in triplicate. No significant difference between total and nonspecific binding was found and the total binding was similar

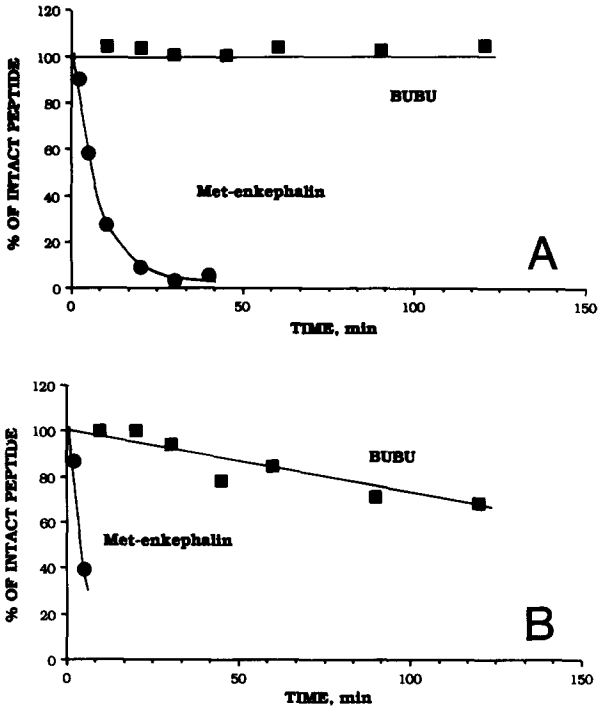

FIG. 1. Degradation kinetics of BUBU and Met-enkephalin by rat serum and rat brain membranes at 37°C. (A) Degradation kinetics of BUBU and Met-enkephalin by rat serum: 10^{-4} M BUBU or Met-enkephalin were incubated during various times with rat serum (6 mg of protein/ml) under a volume of 200 μl. The reaction was stopped by adding 300 μl ethanol. The tubes were centrifuged and the supernatant stored at −80°C. The quantity of nondegraded peptide was measured by HPLC using a C_{18} μ-bondapak column. The elution was carried out with a 25 mM triethylammonium phosphate (pH 6.5) buffer/acetonitrile (63:37) mixture, at a flow rate of 1.2 ml/min. Detection was performed by UV absorption at 210 nm. (B) Degradation kinetics of BUBU and Met-enkephalin by a rat brain membranes preparation: the same procedure was used with rat brain membranes (1.75 mg of protein/ml).

TABLE 1

PERCENTAGES OF INJECTED RADIOACTIVE COMPOUNDS IN THE BRAIN 15 MIN AFTER IV INJECTION TO MICE

Compound (injected dose)	% of Injected Radioactivity in the Brain	% of Injected Tritiated Peptide in the Brain
[^{3}H]DSTBULET (170 pmol)	0.15%	0.0075%
[^{3}H]BUBU (170 pmol)	0.31%	0.065%
[^{3}H]DAGO (170 pmol)	0.40%	0.038%
[^{3}H]Etorphine (80 pmol)	1.8%	

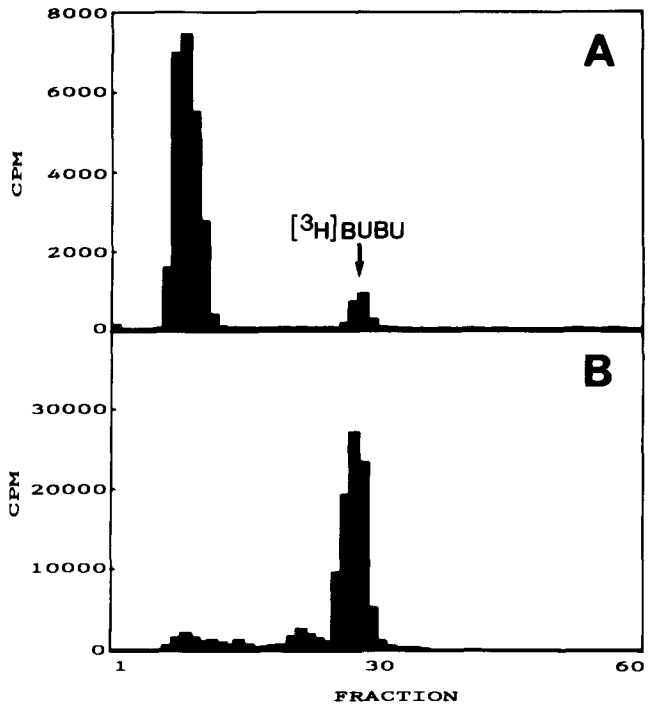

FIG. 2. Chromatogram of the cerebral radioactivity 15 min after IV injection of 170 pmol [^{3}H]BUBU to mice. (A) Treated mouse corresponds to a mouse who has received intravenously 170 pmol [^{3}H]BUBU. The mouse was sacrified 15 min after the injection and the brain quickly removed on ice. The brain was treated in water at 95°C during 5 min, and then homogenized. The homogenate was centrifuged and the supernatant was freeze-dried, dissolved in the buffer used in HPLC and centrifuged. The quantity of radioactivity coeluting with a standard of BUBU was measured by HPLC using a C_{18} μ-bondapak column. The elution was carried out using a linear gradient from 30 to 60% of acetonitrile, in a 25 mM triethylammonium phosphate (pH 6.5) buffer, in 20 min. The flow rate was 1.2 ml/min. The eluate was recovered (1 fraction/30 s) and each fraction was mixed with 5 ml of Biofluor for radioactivity measurement using a 1209 Rackbeta (LKB) counter. (B) Control corresponding to an experiment in which a sample (~5 pmol) of [^{3}H]BUBU was added to a dissected mouse brain immediately prior to homogenization.

in various regions known to contain different concentrations of binding sites such as cerebellum (0.01 fmol/mg of tissue), cortex (0.17 fmol/mg) and striatum (0.24 fmol/mg) (values determined in vitro using 4 nM [^{3}H]BUBU).

The study was therefore carried out after ICV injection of [^{3}H]BUBU, in order to determine the brain concentration of [^{3}H]BUBU required to obtain a significant specific binding. Five doses of [^{3}H]BUBU were injected: 8 pmol, 15 pmol, 30 pmol, 30 pmol with 143 pmol of cold BUBU (isotopic dilution) and 30 pmol with 470 pmol of cold BUBU (isotopic dilution). The nonspecific binding was determined for each dose after coinjection with 3,000 pmol of unlabelled BUBU (3,500 pmol was the maximum that could be injected by this route due to the limited solubility of BUBU). Five mice were used for each determination, and each determination was performed four times. The Scatchard analysis of the specific binding gave the following parameters: $K_{Dapp} = 25 \pm 2$ pmol of injected BUBU; $B_{max} = 16.0 \pm 2.3$ fmol/mg of protein (or 0.45 pmol/brain) (Fig. 3). The percentage of specific binding was 51% after injection of 30 pmol of [^{3}H]BUBU. The bound radioactivity (specific and nonspecific) represented 13% of the total radioactivity present in the brain 15 min after the injection. This total radioactivity accounted for 11% of the ICV injected radioactivity. [^{3}H]BUBU itself (determined by HPLC) represented in the brain 2.4% of the injected dose and 22% of the tritiated material present in the brain.

The IC_{50} value of BUBU on [^{3}H]DSTBULET binding evaluated from the competition curves was of the same order as the apparent K_D value calculated from the saturation experiments, 91 pmol vs. 25. The apparent selectivity ratio (IC_{50}[^{3}H]DAGO/K_{Dapp}) was 78 for BUBU, indicating that under these experimental conditions BUBU was 4.7-fold more selective for δ sites than DTLET (Table 2).

Pharmacological Effects of BUBU and DAGO After IV Administration

BUBU induced a significant increase both in horizontal linear locomotion (at doses of 1 and 3 mg/kg) and vertical rearing activities (only at the dose of 1 mg/kg) (Fig. 4). BUBU and DAGO induced a dose-dependent antinociceptive effect in the mouse hot plate jumping test, 15 min after their IV administration (Fig. 5). The ED_{50} values calculated under these conditions showed that DAGO ($ED_{50} = 3.2$ mg/kg, 95% confidence limits: 1.68–6.08) had a 16-fold greater analgesic potency than BUBU ($ED_{50} = 50$ mg/kg, 95% confidence limits: 39.4–63.5). The difference between the ED_{50} values was greater (52 vs. 16) when maximal antinociceptive responses were measured (5 min after IV administration of DAGO and 10 min after IV administration of BUBU) (1).

DISCUSSION

The aim of this study was to evaluate the resistance of BUBU and DAGO to degrading enzymes and their ability to cross the blood-brain barrier. Therefore, the concentrations of intact [^{3}H]BUBU and [^{3}H]DAGO in the mouse brain were accurately measured in order to evaluate the occupancy of μ and δ receptors at the time of the antinociceptive measurement. Thus, the percentage of injected [^{3}H]BUBU in the brain (0.065%) corresponds to the residual quantity of the intact probe present in the tissue at the time of sacrifice, 15 min after IV administration. This level is weaker than that of [^{11}C]diprenorphine in man (1–2%) (27), but similar to the levels determined for [^{11}C][D.Met^2,Pro^5] enkephalinamide and [^{11}C]Tyr-D.Ala-D.Ala-Phe-Met-NH_2 in monkey (23). However, the degradation of the peptides was not taken into account in this later study. Furthermore, the permeation factor of [^{3}H]BUBU is not very different to those of compounds active at the cerebral level, such as clomipramine (0.1%) (15).

The results obtained in saturation experiments after ICV injection of [^{3}H]BUBU seem to indicate that this probe interacts to a specific and saturable binding site. The specific binding was high, 51% for 30 pmol (a dose close to the K_{Dapp} value) of ICV injected [^{3}H]BUBU per mouse. In these in vivo conditions, the affinity of [^{3}H]BUBU ($K_{Dapp} = 25$ pmol) was similar to that of [^{3}H]DTLET ($K_{Dapp} = 30$ pmol) (31), in good agreement with the affinities determined in vitro. In contrast, the maximal binding capacity of [^{3}H]BUBU ($B_{max} = 0.45$ pmol/brain) was six times lower than that of [^{3}H]DTLET ($B_{max} = 2.5$ pmol/brain) (31). This difference is partly due to the higher selectivity of BUBU as compared to DTLET, and mainly to the more rapid elimination of [^{3}H]BUBU from the brain. Intact [^{3}H]BUBU only represents 2.4% of the ICV injected dose, vs. 8% for [^{3}H]DTLET and [^{3}H]DAGO (31). This probably explains why it was not possible to observe specific binding of [^{3}H]BUBU after IV injection, even using a dose as high as 800 pmol. If we consider that the smallest dose of [^{3}H]BUBU able to give a measurable specific binding (limit of

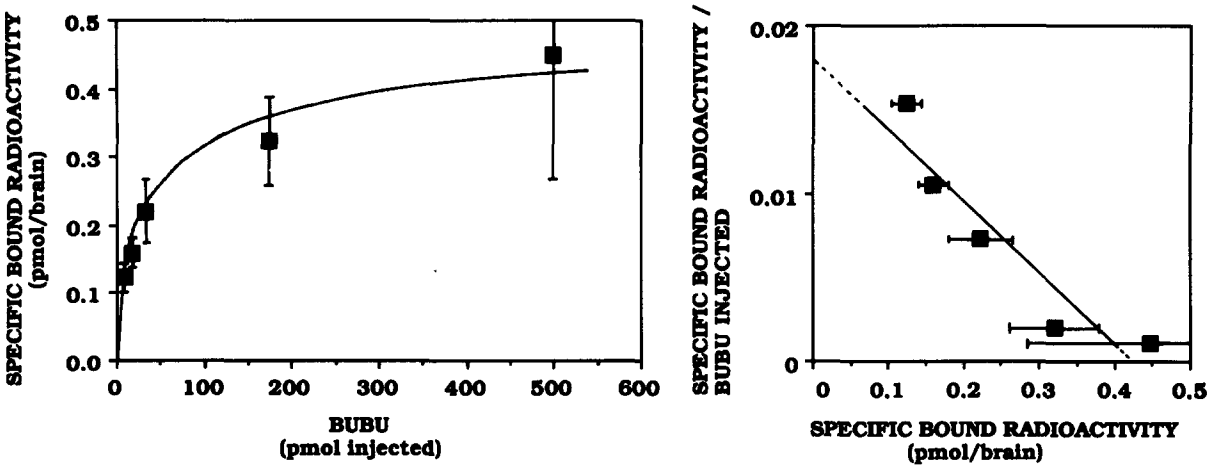

FIG. 3. Specific binding of [^{3}H]BUBU to mouse brain membranes 15 min after ICV injection. Left: the plot represents the saturation curve for [^{3}H]BUBU specifically bound in the brain 15 min after ICV injection vs. the dose injected. Each point represents the mean (±SD) of 5 individual determinations. Specific binding was derived by subtracting from each experimentally determined total binding a calculated value for nonspecific binding. This calculated nonspecific binding was determined, taking into account the quantity of radioactivity remaining in the brain, from the binding observed in mice injected with a dose of [^{3}H]BUBU and 3,000 pmol of unlabelled BUBU. Right: corresponding Scatchard analysis.

sensitivity) is 15 pmol (Fig. 3) and that 0.065% of [^{3}H]BUBU injected IV is present in the brain 15 min after administration, then a very large dose of 23,000 pmol of radiolabelled probe would be necessary to observe any possible discrete distribution. Nevertheless, the pharmacological relevance of the passage of BUBU into the brain of mice and its interaction with opioid receptors is supported both by the increase in spontaneous locomotor activity (significant effects appear at doses of 1 mg/kg) (Fig. 4) as well as the analgesic effect (ED_{50} = 50 mg/kg) that it induced in the hot plate test (Fig. 5).

Local or ICV injection of selective opioids have been carried out to characterize the pharmacological role of each type of opioid receptor (40). However, it was interesting to study the pharmacological effect resulting from the global activation of brain receptors. This study is the first in which the characterization of the responses of μ and δ receptors was carried out after systemic administration, previous works being after local injections of δ agonists (11,32). Systemic administration of BUBU (1 and 3 mg/kg IV) induced an increase in spontaneous locomotor activity. This response was reversed by the δ antagonist naltrindole (1), demonstrating the involvement of the δ type opioid receptor in this effect. It is important to note that a significant effect was obtained at a dose about 50 times lower than that required for antinociceptive responses.

One of the interests of this approach is to allow the relationship between antinociceptive responses observed after IV injection of DAGO and BUBU and the relative μ and δ receptors occupancies (determined from their in vivo binding characteristics) to be studied. The δ receptor occupancy by BUBU at its ED_{50} (50 mg/kg IV) was calculated to be about 97%, while the μ receptor occupancy by DAGO (ED_{50} = 3.2 mg/kg) was calculated to be 44%. The involvement of supraspinal δ receptors in analgesia has been advocated since the highly δ-selective agonist DPDPE elicits antinociceptive responses after ICV administration (26). Furthermore, it has been suggested that morphine interacts with κ and δ sites to induce analgesia when brain μ sites were

TABLE 2

IN VIVO PHARMACOLOGICAL PROFILE OF OPIOID PEPTIDES IN THE MOUSE

	Analgesia ED_{50}, pmol		K_{Dapp} pmol	IC_{50} pmol		% of Receptor Occupancy ICV		IV	
	ICV	IV*		μ	δ	μ	δ	μ	δ
DAGO	9.5†	41	52 ± 15§	54 ± 11§	4,600 ± 1,200§	15.4	0.2	44.1	0.9
DTLET	50‡	—	30 ± 8§	500 ± 133§	133 ± 28§	9.09	64.2	—	—
BUBU	896‡	854	25 ± 2	1,940 ± 740	91 ± 35	31.6	97.3	30.6	97.2

Values are means ± SEM. K_{Dapp} represents the injected dose of the compound necessary to occupy 50% of the total binding sites at the time of sacrifice. The calculation of the % of receptor occupancy has been described in the Method section.

*Expressed in pmol of intact peptide present in the brain.

†Schmidt et al. (unpublished results).

‡From (19).

§From (31).

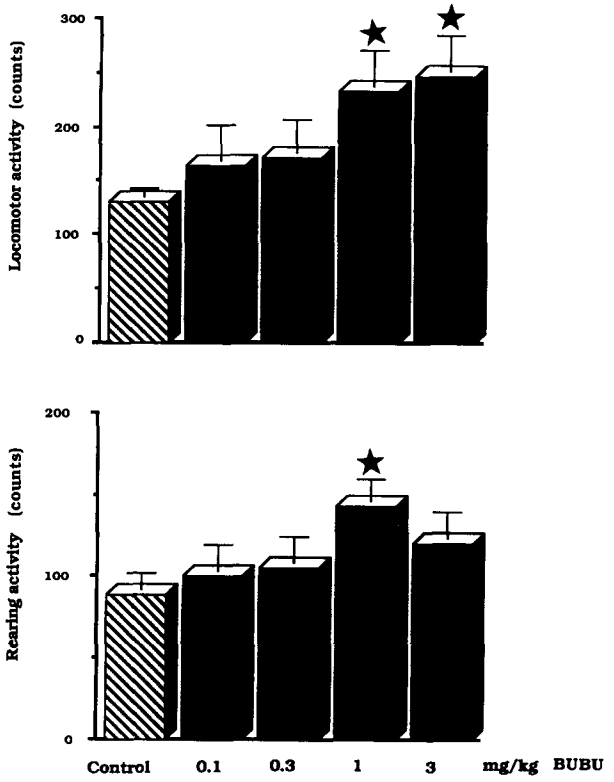

FIG. 4. Locomotor and rearing activities induced by BUBU. The responses were observed 15 min after IV injection of BUBU to mice. Each bar represents the mean ±SEM (n≥10 mice). ⋆$p<0.05$ (Dunnett's t-test).

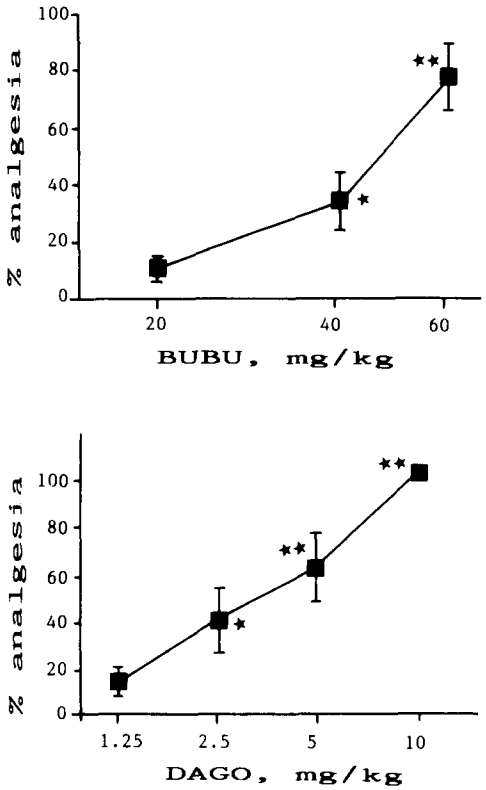

FIG. 5. Analgesic dose-response curves of BUBU and DAGO. The regression lines represent responses seen 15 min after IV administration to mice. The analgesic potency was measured in the hot plate test (jump latency). ⋆$p<0.05$; ⋆⋆$p<0.01$ (Dunnett's t-test).

depleted (25,45) or missing (46). The requirement for a higher δ receptor occupancy (97% vs. 44% for μ sites) could be therefore explained by a higher efficacy of μ vs δ receptors in the control of nociceptive messages at the supraspinal level (38), or to a lower number of δ sites in structures controlling nociceptive messages (13,14). However, several results seem to disagree with the crucial involvement of δ opioid receptors in the control of thermal nociception (hot plate). The δ antagonists ICI 154.129 and ICI 174.864 did not reverse the analgesic effects induced by ICV injection of DTLET and DPDPE (7,9) and the highly selective μ antagonist CTP was shown to block completely the analgesia produced by ICV administration of DPDPE (42). Furthermore, there is a good relationship between the ED_{50} values for ICV injected opioid agonists in various analgesic tests and their apparent affinities for μ binding sites labelled by [^{3}H]DAGO (7, 10, 16). This is confirmed in the present study since, taking into account both the in vivo affinities and cross-reactivities for μ sites of the studied peptides, the in vivo μ receptors occupancy by BUBU after IV administration was about 31% at its ED_{50} (50 mg/kg) and thus very close to that of DAGO at μ receptors (44%) at its ED_{50} (3.2 mg/kg) (Table 2). Identical considerations can be drawn from the calculated occupancy of μ receptors by ICV injected BUBU and DAGO (31.6% for BUBU vs. 15.4% for DAGO) and δ receptors (97.3% vs. 0.2%).

This indicates that highly selective δ agonists could induce antinociceptive responses at very high doses due to their remaining cross-reactivity for μ receptors. This is supported by the inability of the highly selective δ opioid antagonist naltrindole to reverse the analgesia elicited by BUBU and DAGO, while naloxone at low dose was shown to block their analgesic effects (1).

In conclusion, owing to its good bioavailability, [^{3}H]BUBU behaves as a highly selective probe for δ receptors which can be used for in vivo binding experiments. Furthermore, the high brain levels of peripherally administered BUBU means that this agonist can be used to characterize the pharmacological responses elicited by selective global stimulation of δ receptors.

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