3-[^{18}F]Acetylcyclofoxy: a useful probe for the visualization of opiate receptors in living animals

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Received 26 September 1984

A fluoro-analogue of the potent narcotic antagonist, naltrexone, was synthesized and shown to bind with high affinity to opiate receptors in vitro. 3-[^{18}F]acetylcyclofoxy was prepared via a one-step triflate displacement reaction with the positron emitting ^{18}F ion from tetraethylammonium [^{18}F]fluoride. 3-[^{18}F]acetylcyclofoxy accumulation in opiate receptor rich brain regions of both rat and baboon is shown to be completely displaced by the active enantiomer of naloxone ((−)-naloxone) while the identical dose of the pharmacologically inert (+)-naloxone has no detectable effect. Moreover, both rat and baboon brain showed the well documented, typical opiate receptor distribution so that basal ganglia and thalamus are clearly visible in the living baboon brain up to 95 min after intravenous injection of 3-[^{18}F]acetylcyclofoxy. We expect that 3-[^{18}F]acetylcyclofoxy will be a useful probe for visualizing opiate receptors in living humans.

Opiate receptor *PET* *3-[^{18}F]Acetylcyclofoxy* *Stereospecificity* *Naloxone*

1. INTRODUCTION

Pharmacological theory dictates that most drugs bind initially to discrete tissue constituents termed 'receptors' to exert effects in the living animal. In vitro binding of tritiated opiates to rat brain membranes was the basis for the initial demonstration of opiate receptors in brain [1,2]. There is a precise correlation between the in vivo effect of opiate derivatives and the affinity of this binding in vitro over a broad range of pharmacologic potencies [3,4]. A striking demonstration of receptor-relevant biochemical interactions is the high degree of stereoselectivity [5] shown by enantiomeric pairs of opiate agonists such as morphine [6] and the antagonist naloxone [7]. These pairs differ by 1000–10000-fold in binding affinity for the active and inactive enantiomers. Recently, a number of unnatural opiate enantiomers have been synthesized in quantity for the first time [8–10]. These include (+)-naloxone which we use here to demonstrate the stereospecific binding of 3-[^{18}F]acetylcyclofoxy (3) to opiate receptors in the brain of a living baboon.

A great deal is currently known about the distribution of opiate receptors in mammalian brain. Studies of dissected, homogenized brain regions [11,12], as well as autoradiographic [13,14] patterns of opiate receptor distribution on thin sections of rat brain consistently show high levels of radiolabeled opiate-binding sites in the caudate nucleus/basal ganglia, thalamus, and prefrontal/frontal cortex with undetectable binding in the cerebellum. Moreover, dissection [15] or visualization [16] of intravenously injected radiolabeled opiates also demonstrates a similar pattern of anatomical localization. We now show that 3-[^{18}F]acetylcyclofoxy binds in vivo in rat and ba-

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Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

boon in the expected opiate receptor patterns in a stereospecific manner.

Opiates containing positron emitting radionuclides have been proposed as suitable probes for detecting opiate receptors in living humans [17–19]. However, 3-[^{18}F]acetylcyclofoxy is the first positron emitting opiate for which the active and inactive forms of naloxone were used to unequivocally demonstrate stereospecific displacement from opiate receptor-rich regions. 3-[^{18}F]Acetylcyclofoxy thus fulfills the dual criteria of stereospecific displacement and concordance with the previously demonstrated neuroanatomical pattern of opiate receptor distribution in rat and primate brain.

2. MATERIALS AND METHODS

2.1. *Chemistry*

3-Acetyl-6-deoxy-6-β-[^{18}F]fluoronaltrexone (3-[^{18}F]acetylcyclofoxy (3)) was synthesized (fig.1) from the known 3-acetyl-6-α-naltrexol (1) via the triflate 2. The latter was prepared by treatment of (1) with trifluoromethanesulfonic anhydride in chloroform containing excess pyridine as described in [20]. ^{18}F was produced from $^{6}Li_2CO_3$ using the high flux reactor (1.1×10^{14} n/cm^2 per s) at the National Bureau of Standards. The reaction of triflate 2 with $Et_4N^{18}F$ in anhydrous acetonitrile containing Et_4NOH at 80°C for 0.25 h provided 3-[^{18}F]acetylcyclofoxy (3). The product was purified on reversed-phase HPLC eluted with 55% MeOH, 45% H_2O with 5 mM octanesulfonic acid at pH 3 to give a 30% radiochemical yield uncorrected for decay. The lower limit of the effective specific activity at the time of injection was approximately 20 Ci/mmol assuming that all chemical impurities have the same extinction coefficient and the same opiate receptor affinity constant (see [20]). By Scatchard analysis of 3-[^{18}F]acetylcyclofoxy (3) and (−)-[^{3}H]naloxone binding to rat brain membranes the specific activity was estimated to be approx. 50 Ci/mmol.

2.2. *Biochemistry*

Stereospecific opiate receptor binding to rat brain membrane in vitro was measured with non-radioactive (+)- or (−)-naloxone (10^{-6} M) and (−)-[^{3}H]naloxone (New England Nuclear) on [^{3}H]Foxy ([^{3}H]-5) [21] as described. Accumulation of injected 3-[^{18}F]acetylcyclofoxy in vivo was measured by scraping approximately equivalent areas of anatomically distinct horizontal sections (24 microns) (fig.2a) into detergent fluor, sonicating and counting at 90% efficiency by liquid scintillation spectrophotometry. The image of 3-[^{18}F]acetylcyclofoxy accumulation in cryostat-cut 120 micron horizontal brain sections was obtained from a rat injected in vivo with 30 μCi 3-[^{18}F]acetylcyclofoxy and sacrificed 30 min later by decapitation; the section was juxtaposed overnight against Kodak S20 film and developed in the standard way the following morning (fig.2b).

2.3. *Imaging procedure*

The role of opiate receptor binding in the anatomic localization of 3-[^{18}F]acetylcyclofoxy was studied using (+)-naloxone (6) (inactive) and (−)-naloxone (7) (active) (fig.3). A 16 kg male baboon was studied on 2 successive days: August 30 and 31, 1984. The animal was sedated with 1.5 mg/kg ketamine and 0.4 mg/kg Ropum and intubated. He was placed supine on a rigid table for the PET tomography, allowed to breath 40% oxygen and maintained under anesthesia by a continuous i.v. infusion of 4.8–6 mg/kg per h sodium pentothal. On each imaging day, after the animal's vital signs had stabilized, a rapid bolus injection of 340 μCi 3-[^{18}F]acetylcyclofoxy (3) was made. The anesthetized baboon was positioned identically on both days in the ring of a high-resolution positron emission tomograph, the Neuro PET [22].

The experimental sequence was identical on both days, with the exception that just prior to image 17, (−)-naloxone was injected on August 30, and saline injected August 31. The following experimental sequence was employed: image 1–5, 1 min per image; image 5–10, 5 min per image; image 11–19, 10 min per image. Just prior to image 11, saline was given; prior to image 14, (+)-naloxone was given and prior to image 17, saline (August 31) or (−)-naloxone (August 30) was given. At each image collection period, 5 tomographic slices were obtained parallel to the canthomeatal line with the position of the slices at 37 mm below, to 20 mm above the c-m line. Peak count rates obtained were about 10000 cps for all slices. The attenuation coefficient was calculated by considering each slice to be an ellipse defined by a region of interest drawn just external to the scalp

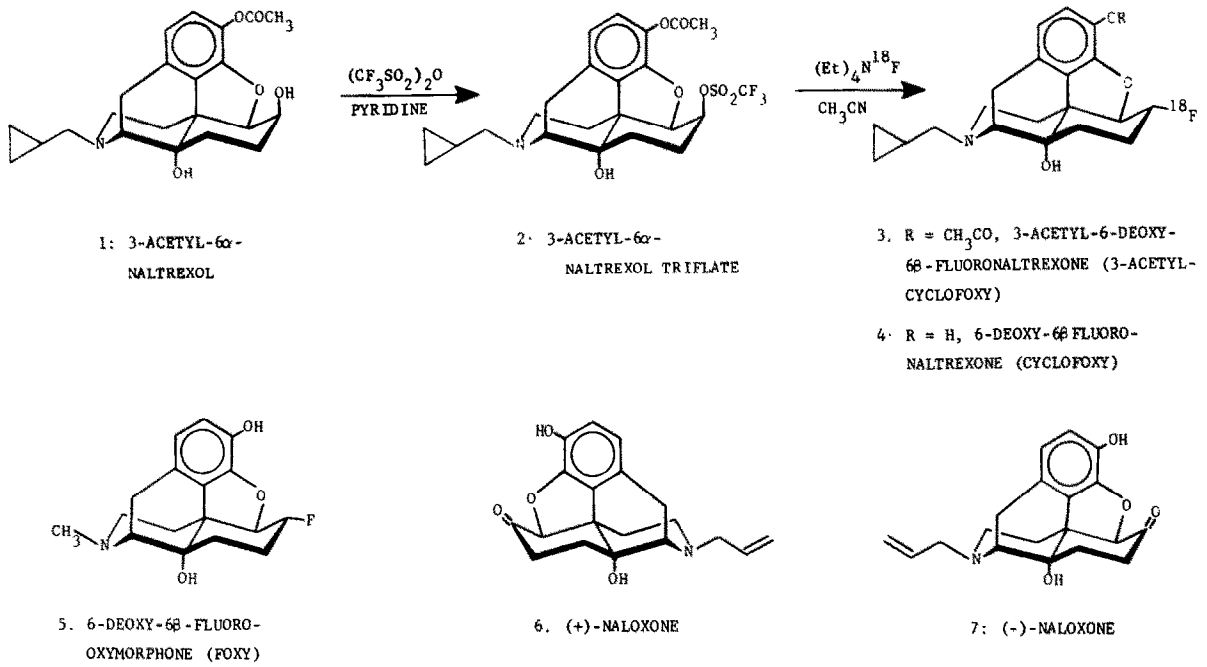

Fig.1. Synthesis of 3-[^{18}F]acetylcyclofoxy (3); structures of foxy (5) and (+)- and (−)-naloxone (6 and 7, respectively).

seen on early view. Image reconstruction was performed using a filtered back-projection technique. Attenuation corrected, reconstructed images where photographed from the CRT screen onto transparency film using a Dunn Photomat, at each imaging interval.

3. RESULTS

Fig.1 shows the route of chemical synthesis for [^{18}F]acetylcyclofoxy (3), as well as structures for the enantiomers (+)- and (−)-naloxone (6 and 7, respectively), and foxy (5), the agonist analogue of cyclofoxy which, as expected from previous reports [23], fails to accumulate in vivo. Synthesis of the highly reactive triflate precursor of 3-acetylcyclofoxy provided a rapid, one-step radiochemical labeling procedure. We chose introduction of the fluoro substitution at the 6 position of the morphinan ring system since this position is easily manipulated in 1 and such modifications generally give active compounds [24].

Cyclofoxy [20] as expected, at 2 nM retained the high affinity of its prototype naltrexone (1 nM) in in vitro binding experiments. 3-[^{18}F]Acetylcyclofoxy binds in vitro stereospecifically to opiate receptor membrane preparations. Horizontal brain sections of rats injected with 3-[^{18}F]acetylcyclofoxy showed an accumulation pattern in vivo (table 1, fig.2b) quite similar to that of in vitro stereospecific [^{3}H]naloxone binding (fig.2a). Moreover, (−)-naloxone (1 mg/kg) completely blocked regional brain accumulation. The same

Table 1

Comparison of 3-[^{18}F]acetylcyclofoxy (22.6 μCi/220 g rat) accumulation in several regions of brains of rats co-injected with (−) or (+) non-radioactive naloxone (1 mg/kg) and killed after 30 min

Region[b]	(−)-Naloxone	(+)-Naloxone
	(cpm/72 μm slice)	
Cerebellum	64 ± 9	47 ± 8
Colliculi	58 ± 10	134 ± 12[a]
Thalamus	78 ± 11	260 ± 22[a]
Caudate	80 ± 9	214 ± 18[a]

[a] Values represent X ± SE determined in quintuplicate for each of 2 rats

[b] As diagrammed in fig.2A

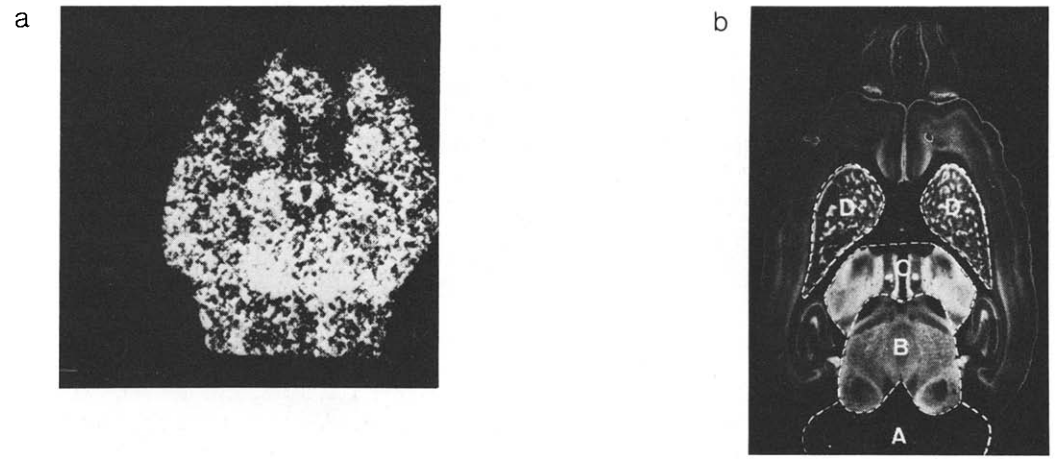

Fig.2. (a) An autoradiograph of a horizontal section (24 microns) of rat brain with in vitro labeling of opiate receptors (in white) with [^{3}H]naloxone illustrates the plane of dissection used to obtain table 1 data [13]. (b) An image-enhanced autoradiograph of a horizontal section (120 μm) of brain from a rat injected with 3-[^{18}F]acetylcyclofoxy (see section 2). The very short (110 min) half-life of ^{18}F necessitated a thick brain section to obtain this low-resolution image.

dose of (+)-naloxone had no effect. We constantly monitored the relative specific activity ($N = 5$) by measuring the caudate:cerebellum ratio of accumulation (5–8:1) after 3-[^{18}F]acetylcyclofoxy injection.

An anesthetized baboon injected with 3-[^{18}F]acetylcyclofoxy showed the striking and dramatic pattern expected for opiate receptor distribution (fig.3). Moreover, a low dose of (−)-naloxone (0.13 mg/kg) completely abolished this

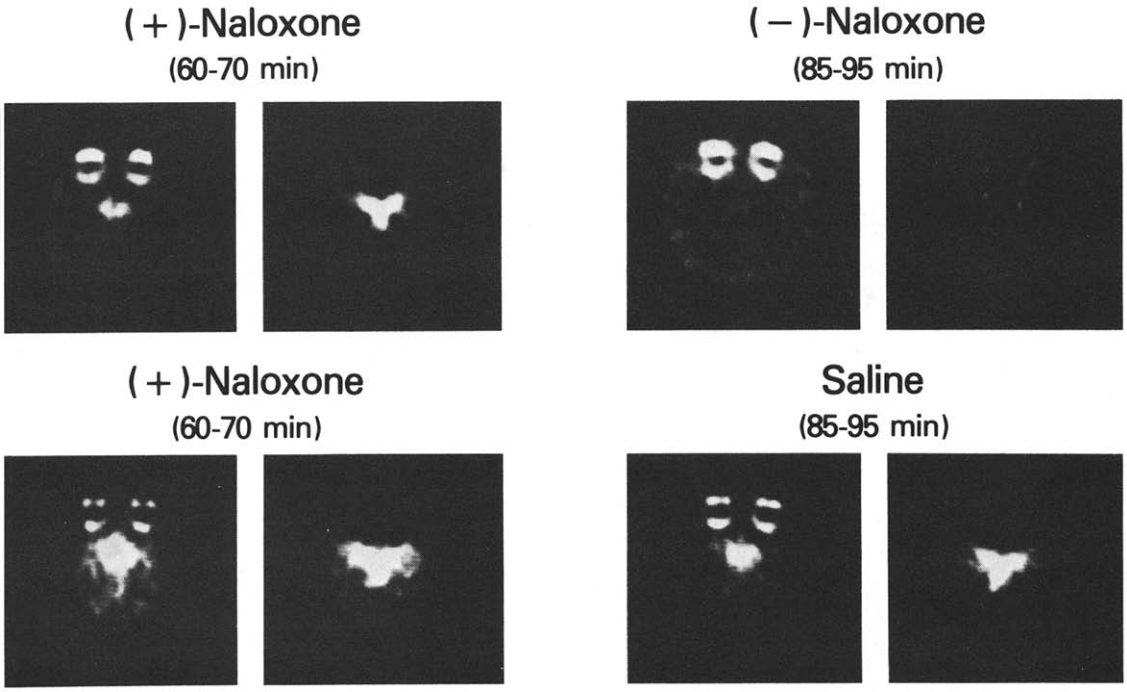

Fig.3. Sequential positron emission tomography in a living baboon after intravenous injection of 3-[^{18}F]acetylcyclofoxy and subsequent injections of (+)- or (−)-naloxone (0.13 mg/kg). Sections at eye level show receptor unrelated non-displaceable ^{18}F accumulation by melanocytes in the retina [31].

pattern, while the identical dose of (+)-naloxone injected before a previous 10-min time segment did not alter the pattern. The following day, once again it was shown that (+)-naloxone failed to reduce the striking pattern of opiate receptor distribution in baboon brain, since 3-[^{18}F]acetylcyclofoxy is still clearly visible in the basal ganglia and thalamus at 95 min post-injection (fig.3).

4. DISCUSSION

Generally, while psychoactive drugs always bind in vivo to their brain receptors, their accumulation in receptor-rich areas can seldom be measured over the 'noise' of unbound drug. Studies of bound *vs* unbound radiolabeled opiates accumulated in regions of rat brain at various times after intravenous injection have revealed that the percentage of receptor-mediated accumulation increases with time [15]. We show that 3-[^{18}F]acetylcyclofoxy is a useful probe for visualizing stereospecific opiate receptor binding in vivo; both the rat slice and baboon brain images clearly display the expected opiate receptor pattern. Initially, the 3-[^{18}F]acetylcyclofoxy is distributed to all regions by blood flow. We observed rapid efflux of radioactivity from many opiate receptor-poor regions such as cerebellum and occipital cortex. In contrast, 3-[^{18}F]acetylcyclofoxy was retained in the basal ganglia and thalamus.

Since probes with highest receptor affinity are most useful for visualizing receptors, opiate antagonists accumulate much more readily than their agonist analogs [23] in the sodium-rich in vivo environment. Thus, it is clear that 3-[^{18}F]acetylcyclofoxy is an antagonist. [^{3}H]Foxy, its agonist analog, shares a high in vitro (sodium-free) affinity in the 1–2 nM range [21] but fails to accumulate in vivo (caudate:cerebellum = 1). Previously documented in vivo binding has been reported for antagonists only, including [^{3}H]naloxone [15] and [^{3}H]diprenorphine [16].

Acetylation in the 3 position, e.g., heroin, insures an efficient initial delivery to brain (about 5% *vs* morphine's less than 0.1%). Interestingly, by analogy with the rapid deacetylation of heroin in vivo [25], phenolic cyclofoxy is probably the active species in brain. Foxy is an acronym for 5, a fluoro derivative of oxymorphone; we refer to the *N*-cyclopropylmethyl analog 4 as cyclofoxy.

A recent paper [26] suggests that region-specific opiate receptor binding in vivo is susceptible to blockade by the endogenous opiate peptides (i.e., endorphins/dynorphins/enkephalins) released by environmental stimuli. This raises the exciting possibility that the functional aspects of opiatergic neurocircuitry in individuals might be assessed if 3-[^{18}F]acetylcyclofoxy, as expected, is suitable for human use. Fortunately, 3-[^{18}F]acetylcyclofoxy appears to label the rather interesting μ (antagonist) conformation of the type I opiate receptor complex. These opiate receptors show an exponential increase along monkey cortex from the visual cortex (back of brain) to the frontal cortex, a cortical distribution pattern corresponding to the increasing complexity of sensory information processing occurring along this gradient [27]. Here (image not shown), the frontal cortex of the living baboon was apparently labelled with 3-[^{18}F]acetylcyclofoxy; it is exciting to contemplate studies of the larger human cortex where the neuronal macromolecules we call opiate receptors, which have appeared relatively recently in evolution [28], are densest in those expanded regions [29,30] serving 'higher' functions.

ACKNOWLEDGEMENTS

The authors appreciate the valuable assistance of Dr Richard Carson, Mr Paul Baldwin, Ms Carol Kowolski, Ms Ann Tran, Ms Camille Vermess, Mr Ernest Owens and Dr Stafford McLean.

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