Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 459 (2003) 217–219
www.elsevier.com/locate/ejphar

Short communication

Naloxone displacement at opioid receptor sites measured in vivo in the human brain

Jan K. Melichar, David J. Nutt*, Andrea L. Malizia

Psychopharmacology Unit, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK

Received 12 November 2002; received in revised form 2 December 2002; accepted 6 December 2002

Abstract

We report the use of a sensitive non-tomographic positron detecting system to measure the dose–response curve of naloxone in human brain. [^{11}C]Diprenorphine was administered to normal volunteers in tracer amounts and, 30 min later, various bolus doses of naloxone were given (1.5–160 μg/kg) intravenously and change in [^{11}C]diprenorphine binding monitored over the next 30 min. We found that this method produced results consistent with existing data. It was observed that ~13 μg/kg of naloxone (~1 mg in an 80 kg man) was required to produce an estimated 50% receptor occupation. This is consistent with the clinical dose of naloxone used to reverse opiate overdose (0.4 mg–1.2 mg).

Keywords: Opioid receptor; Opioid receptor antagonist; Diprenorphine; Naloxone; PET (Positron-Emission Tomography)

1. Introduction

Drugs active at the opioid receptor are important in a variety of clinical conditions, as well as in the field of addiction. Measures of opioid receptor binding and occupancy in man in vivo can be obtained using [^{11}C]diprenorphine, a positron-emitting ligand. This has been used with Positron-Emission Tomography (PET) to delineate the distribution of opioid receptors in the human brain (Jones et al., 1988) and in the study of a variety of clinical conditions, including epilepsy (Bartenstein et al., 1993) and pain (Jones et al., 1999). [^{11}C]Diprenorphine is a weak partial opiate agonist which is structurally similar to naloxone (a full opiate antagonist) and labels μ, κ and δ brain opioid receptors with similar affinities (Jones et al., 1988; Sadzot et al., 1991; Seeman, 1993). In conventional PET scans, it takes 20–30 min for [^{11}C]diprenorphine to reach maximal levels in the brain, with a stable level of activity or slow decline being observed in opioid receptor-rich regions (e.g. frontal, temporal and parietal cortices) in the following 30 min.

The Multiple Organs Coincidences Counter (MOCC) is a non-tomographic alternative to PET. It is a whole body gamma-ray counter modified to detect coincident counts from whole regions of the body and is very sensitive, detecting radiolabelled tracers given at <1% of the dose used in PET. The modification measures coincident counts between pairs of highly sensitive sodium iodide detectors and gives a field of view of approximately 10 cm. Thus, the left chest is used, where necessary, as a reference region, as it is not possible to delineate separate areas of the brain with this instrument. Reproducible time activity curves of exactly the same temporal profile to those from conventional PET can be obtained from whole body areas exposing patients to 50–100 μSv of radiation as opposed to 1.5–2.5 mSv with conventional PET (Malizia et al., 1995).

Naloxone is a full opiate antagonist and is used clinically for the complete or partial reversal of narcotic depression, including respiratory depression, induced by opioids including natural and synthetic narcotics. It is also indicated for the diagnosis of suspected acute opioid overdosage, where an initial dose of 0.4 mg to 1.2 mg is usually administered intravenously (occasionally up to 10 mg is given).

This study aims to validate the use of pulse chase (or displacement) experiments in the MOCC to measure naloxone occupation in the human brain and to assess the relationship between naloxone occupation and the doses required in clinical use. Previous work in the MOCC has validated this paradigm using the tracer flumazenil to measure $GABA_A$ receptor sites (Malizia et al., 1995). Villemagne et al. (1994) have previously conducted studies on the opioid receptors

* Corresponding author. Tel.: +44-117-925-3066; fax: +44-117-927-7057.
E-mail address: david.j.nutt@bristol.ac.uk (D.J. Nutt).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(02)02872-8

using a similar instrument, though these studies used an equilibrium 'preloading' paradigm. The advantage of the 'displacement' paradigm used in the present study is that all the data can be collected in a single experiment. Calculation of occupancy measures may be biased by changes in tissue perfusion. Naloxone has been reported in particular conditions to change brain blood flow (Nagamachi et al., 1995; Komjati et al., 2001).

2. Materials and methods

Five healthy volunteers were studied in the MOCC, receiving an initial injection of ~80 μCi of [^{11}C]diprenorphine. The activity was recorded from the head and the left chest for 1 h after injection (the maximum possible, given the short half life of [^{11}C]diprenorphine and the low radiation doses given), and analysed as previously described (Malizia et al., 1995; Melichar et al., 2001). Thirty minutes after the initial injection, they received a bolus i.v. injection of naloxone and the change in gradient of the washout curve before and after administration of naloxone was measured. Three volunteers had only one study, one had a further two studies and another had a further three studies (in these two individuals, they received different doses of naloxone in each study and in one of their studies received no naloxone). Thus, in total, two control studies and eight naloxone displacement studies were done. Doses of naloxone given were 1.5, 4, 5, 10, 12.5, 15, 80 and 160 μg/kg. For an 80-kg man, this corresponds to 0.12, 0.32, 0.4, 0.8, 1, 1.2, 6.4 and 12.8 mg in total, respectively. The protocol was approved by the Administration of Radioactive Substances Advisory Committee (ARSAC) and the local Ethics Committee.

3. Results

The signals from the head were much greater than those from the left chest and demonstrated an increased [^{11}C]diprenorphine washout rate after injection of naloxone (see Fig. 1), which continued until the end of the study (a further 30 min). This change in washout rate was measured and the results are shown in Fig. 2 in the traditional log dose–response format. From this, it is estimated that 50% of opioid receptors in the brain are occupied by naloxone at a dose of approximately 13 μg/kg.

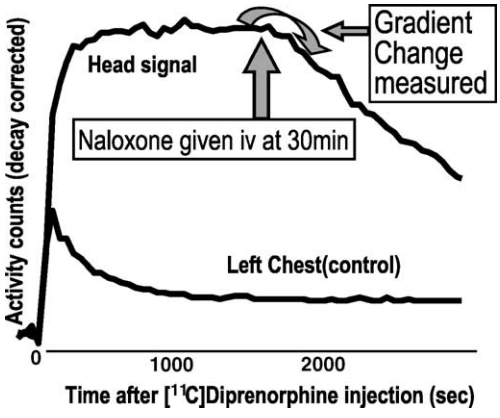

Fig. 1. Typical [^{11}C]diprenorphine MOCC study, illustrating significant reduction of signal when naloxone bolus given.

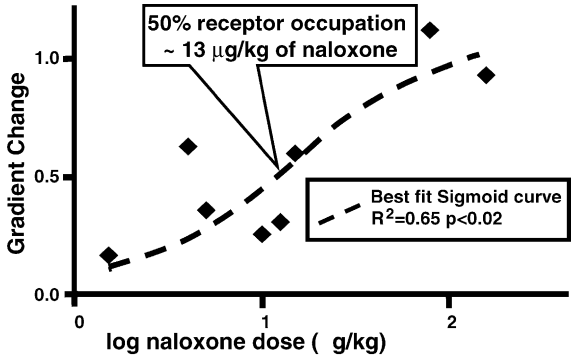

Fig. 2. Log dose–response curve: naloxone displacement of [^{11}C]diprenorphine in the MOCC in eight healthy volunteers—gradient changes.

4. Discussion

These results demonstrate that this method is useful for calculating opioid receptor occupation in man in vivo. We have shown that the dose needed to occupy 50% of available receptors in the adult human brain is approximately 13 μg/kg, which, in an 80-kg man, corresponds to 1.04 mg. This closely mirrors the doses given in clinical practice for the treatment of opioid overdose (usually 0.4 to 1.2 mg, i.e. one to three ampoules at 0.4 mg/ampoule). These data are consistent with previous observations by Villemagne et al. (1994) who used a preloading paradigm. We can therefore conclude that a displacement paradigm seems effective in healthy volunteers. It can also be inferred that to block the effects of an overdose of opioid agonists with naloxone requires around 50% blockade of available opioid receptors, given the results from this study and everyday clinical practice with naloxone.

However, this study does not tell us what occurs in the brains of opiate-dependent individuals—further experiments will be needed before extrapolating these results to this group. This is because these individuals may well have changes in receptor numbers due to their chronic misuse of opiates and might exhibit large changes in cerebral blood flow when exposed to naloxone (Zamani et al., 2000).

In summary, this is a valid technique for deriving an index of opioid receptor site occupation using very low doses of radiation which allows repeated measurements to be made. We have shown that this method estimates 50% receptor occupation by ~13 mg/kg of naloxone in man in vivo, which is in line with both pre-clinical work and clinical experience.

Acknowledgements

We are grateful for the help and support of the staff at the MRC Cyclotron Centre in London, especially Dr. Roger Gunn, as well as Dr. Judy Myles, Dr. Anne Lingford-Hughes and Dr. Mark Daglish in Bristol and to the Wellcome Trust and the Medical Research Council for the financial support.

References

Bartenstein, P.A., Duncan, J.S., Prevett, M.C., Cunningham, V.J., Fish, D.R., Jones, A.K., Luthra, S.K., Sawle, G.V., Brooks, D.J., 1993. Investigation of the opioid system in absence seizures with positron emission tomography. J. Neurol. Neurosurg. Psychiatry 56, 1295–1302.

Jones, A.K., Luthra, S.K., Maziere, B., Pike, V.W., Loc'h, C., Crouzel, C., Syrota, A., Jones, T., 1988. Regional cerebral opioid receptor studies with [^{11}C]diprenorphine in normal volunteers. J. Neurosci. Methods 23, 121–129.

Jones, A.K., Kitchen, N.D., Watabe, H., Cunningham, V.J., Jones, T., Luthra, S.K., Thomas, D.G., 1999. Measurement of changes in opioid receptor binding in vivo during trigeminal neuralgic pain using [^{11}C]diprenorphine and positron emission tomography. J. Cereb. Blood Flow Metab. 19, 803–808.

Komjati, K., Greenberg, J.H., Reivich, M., Sandor, P., 2001. Interactions between the endothelium-derived relaxing factor/nitric oxide system and the endogenous opiate system in the modulation of cerebral and spinal vascular CO2 responsiveness. J. Cereb. Blood Flow Metab. 21, 937–944.

Malizia, A., Forse, G., Haida, A., Gunn, R., Melichar, J., Poole, K., Bateman, D., Fahy, D., Schorr, L., Brown, D., Rhodes, C., Nutt, D., Jones, T., 1995. A new human (psycho)pharmacology tool: the multiple organs coincidences counter (MOCC). J. Psychopharmacol. 9, 294–306.

Melichar, J.K., Haida, A., Rhodes, C., Reynolds, A.H., Nutt, D.J., Malizia, A.L., 2001. Venlafaxine occupation at the noradrenaline reuptake site: in-vivo determination in healthy volunteers. J. Psychopharmacol. 15, 9–12.

Nagamachi, K., Shitara, K., Yamashita, Y., Morita, H., Nishida, Y., Maeta, H., Tanaka, S., Hosomi, H., 1995. Role of endogenous opioids and central opioid receptors in cerebral cortical blood flow autoregulation. Jpn. J. Physiol. 45, 137–149.

Sadzot, B., Price, J.C., Mayberg, H.S., Douglass, K.H., Dannals, R.F., Lever, J.R., Ravert, H.T., Wilson, A.A., Wagner Jr., H.N., Feldman, M.A., 1991. Quantification of human opiate receptor concentration and affinity using high and low specific activity [^{11}C]diprenorphine and positron emission tomography. J. Cereb. Blood Flow Metab. 11, 204–219.

Seeman, P., 1993. Receptor tables. Drug Dissociation Constants for Neuroreceptors and Transporters, vol. 2. SZ Research, Toronto.

Villemagne, V.L., Frost, J.J., Dannals, R.F., Lever, J.R., Tanada, S., Natarajan, T.K., Wilson, A.A., Ravert, H.T., Wagner Jr., H.N., 1994. Comparison of [^{11}C]diprenorphine and [^{11}C]carfentanil in vivo binding to opiate receptors in man using a dual detector system. Eur. J. Pharmacol. 257, 195–197.

Zamani, R., Semnanian, S., Fathollahi, Y., Hajizadeh, S., 2000. Systemic naloxone enhances cerebral blood flow in anesthetized morphine-dependent rats. Eur. J. Pharmacol. 408, 299–304.