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Effects of cocaine on [^{11}C]norepinephrine and [^{11}C]β-CIT uptake in the primate peripheral organs measured by PET

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The toxic properties of cocaine are related to both the central and peripheral effects. To identify possible lethal mechanisms and the accumulation of cocaine in various organs, the effects of cocaine on [^{11}C]norepinephrine and cocaine congener [^{11}C]β-CIT uptake in Cynomolgus monkeys were measured by positron emission tomography (PET). Cocaine (5 mg/kg) noticeably inhibited [^{11}C]norepinephrine uptake in the heart. The uptake of [^{11}C]β-CIT in the heart and lung was reduced by pretreatment with cocaine. There was a significant uptake in the liver which was increased following cocaine pretreatment. The results of this study confirm that cocaine blocks the neuronal uptake of norepinephrine in sympathetic nerve terminals in the myocardium. The effect of cocaine on [^{11}C]β-CIT uptake indicates that the binding sites in the heart and lung are saturable, while the uptake mechanism in the liver is different from those of the heart and lung.

Key words: [^{11}C]β-CIT, [^{11}C]norepinephrine, PET, cocaine, peripheral organs

INTRODUCTION

THE LETHAL EFFECTS of cocaine are commonly seen in the population of abusers. The mechanism of the lethality has been ascribed to effects on the central nervous system and peripheral organs, but the mechanism of lethality remains to be clarified.

The uptake of ^{11}C-labeled cocaine in peripheral organs has been examined by positron emission tomography (PET),[1,2] but the potential for the detection of binding to specific sites is limited by the low affinity of [^{11}C]cocaine for the recognition sites.[2,3] An alternative to a direct study of [^{11}C]cocaine binding in man is to examine how unlabeled cocaine inhibits the binding of suitable radio-ligands. The cocaine congener β-CIT (2β-carbomethyoxy-3β-[4-iodophenyl]tropane) has high affinity for the monoamine transporters,[4] and marked accumulation has been demonstrated in peripheral organs such as the heart, lung and liver.[5–7] β-CIT, labeled with carbon-11,[3] has been used to characterize the binding site in the brain.[8]

Inhibition of norepinephrine reuptake has been suggested as one reason for the lethality of cocaine. Racemic norepinephrine has recently been labeled with carbon-11[9] and used as a ligand to visualize the uptake in sympathetic nerve terminals of primate myocardium.[10] Accordingly, the effect of cocaine on [^{11}C]norepinephrine uptake can be directly demonstrated by PET.

The aim of the present PET study was to measure the effect of unlabeled cocaine on the uptake of both [^{11}C]norepinephrine and [^{11}C]β-CIT in primate peripheral organs and to examine the possible toxic effects and the distribution of cocaine recognition sites.

METHODS

This study was performed at the Department of Clinical Neuroscience at the Karolinska Hospital, Stockholm, Sweden.

Chemistry

Racemic [^{11}C]norepinephrine was prepared from

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[^{11}C]nitromethane.[9] β-CIT was labeled with ^{11}C by N-methylation of nor-β-CIT with [^{11}C]methyl iodide.[3] The radiochemical purity was higher than 99% and the specific radioactivity was about 37 TBq/mmol at the time of injection.

PET system

The PET system (Scanditronix PC2048-15B) measures radioactivity in 15 horizontal sections and a spatial resolution in the imaging plane of about 4.5 mm FWHM (full width at half maximum). The attenuation correction was carried out with a transmission scan. Radioactivity in the monkey chest and upper abdomen was measured according to a pre-programmed sequence of frames until 30–60 min after injection of [^{11}C]β-CIT or [^{11}C]norepinephrine.

Cynomolgus monkeys

Three male Cynomolgus monkeys (4.0–4.5 kg) were obtained from the National Laboratory of Bacteriology, Solna, Sweden.

Anesthesia was induced by repeated i.m. injection of ketamine (Ketalar® 5–10 mg kg^{-1}h^{-1}). During the PET study the position of the monkey chest was fixed with a plastic holder.

PET experiments with [^{11}C]norepinephrine

The pretreatment experiment was performed two hours after the baseline experiment. A sterile physiological phosphate buffer (pH 7.4) solution containing [^{11}C]norepinephrine (10–13 MBq) was injected as a bolus into a sural vein. Cocaine (5 mg/kg) was injected intravenously 5 min before the second injection of [^{11}C]norepinephrine. This dose of cocaine was chosen based on the fact that 2 mg/kg and 7 mg/kg displaces 20% and 50% of the radioactivity of [^{11}C]β-CIT in the striatum.[8]

PET experiments with [^{11}C]β-CIT

Two baseline experiments and two pretreatment experiments were performed. A sterile physiological phosphate buffer (pH 7.4) solution containing [^{11}C]β-CIT (34–39 MBq) was injected as a bolus into a sural vein. Cocaine (5 mg/kg) was injected intravenously 5 min before the second injection of [^{11}C]β-CIT.

Data analysis

Regions of interest (ROIs) were drawn on the reconstructed PET images. Irregular ROIs were drawn for the left ventricular myocardium and septum, the right lung and the middle area of the liver. Regional radio-activity was determined for each frame and corrected for decay. The injected radioactivity was normalized to 37 MBq and regional radioactivity was plotted versus time.

The area under the radioactivity curve (AUC) obtained after the injection of [^{11}C]β-CIT was calculated by the KaleidaGraph™ program on a Macintosh computer. The interval from 1 to 30 min was used for the lung and heart.

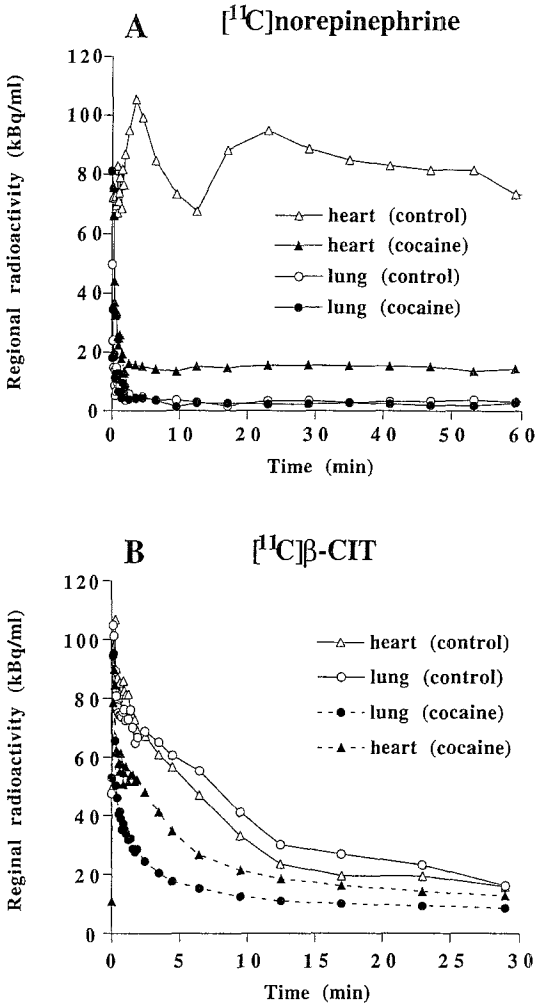

Fig. 1 Time course for regional radioactivity (kBq/ml) in the heart and lung of a Cynomolgus monkey after i.v. injection of (A) [^{11}C]norepinephrine and (B) [^{11}C]β-CIT. Control (open symbols) and pretreatment experiment with cocaine (5 mg/kg) (filled symbols). The injected radioactivity was normalized to 37 MBq and plotted versus time.

The effect of cocaine was expressed as the percentage of the AUC obtained in the control study.

RESULTS

[^{11}C]norepinephrine

In the control study, there was a noticeable uptake of radioactivity in the heart (Fig. 1A). During the initial 20 min, movement artifacts occurred. The radioactivity of the myocardium was more than ten times that in the adjacent lung tissue. Following pretreatment with cocaine (5 mg/kg), the uptake of radioactivity in the myocardium was noticeably reduced (Fig. 1A). In the lung, there was no evident effect of cocaine (Fig. 1A).

[^{11}C]β-CIT

In the control studies, there was a high and transient initial peak in lung radioactivity (Fig. 1B). The uptake in the

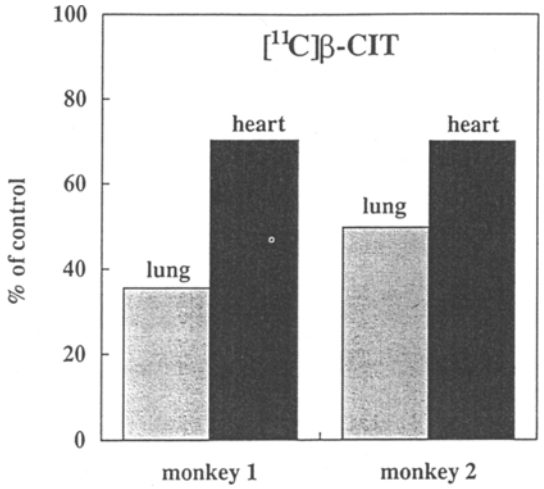

Fig. 2 The relative uptake of [^{11}C]β-CIT in the heart and lung of a Cynomolgus monkey after pretreament with cocaine (5 mg/kg). Data are expressed as percentage of the respective control. The values were calculated as the area under the time activity curve (AUC) from 1 to 30 min after the injection of [^{11}C]β-CIT.

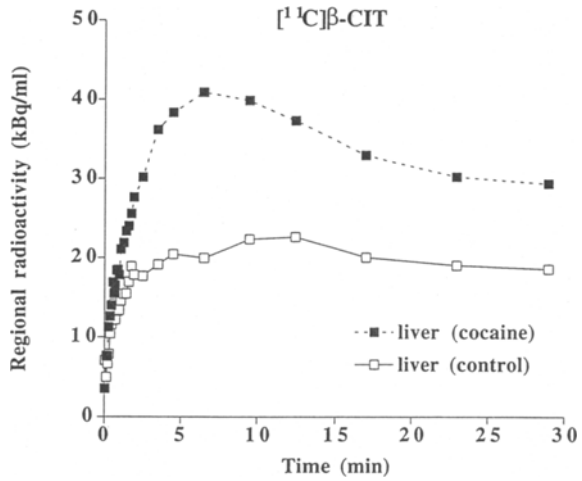

Fig. 3 Time course for regional radioactivity (kBq/m*l*) in the liver of a Cynomolgus monkey after i.v. injection of [^{11}C]β-CIT. Control (open symbols) and pretreatment with cocaine (5 mg/kg) (filled symbols). The injected radioactivity was normalized to 37 MBq and plotted versus time.

heart and lung was at a similar level (Fig. 1B). Figure 2 shows the relative uptake in the lung and heart following pretreatment compared to that in the control study. In the two experiments with cocaine (5 mg/kg), the uptake in the heart was reduced by 30% and the uptake in the lung was reduced by 50–65%.

The radioactivity in the liver increased slowly and reached a peak after about 12 min (Fig. 3). The hepatic uptake was increased by pretreatment with cocaine.

DISCUSSION

A recent PET study[10] demonstrated that [^{11}C]norepinephrine is suitable for the visualization of neuronal uptake, referred to as uptake-1.[11] In the present study, pretreatment with cocaine (5 mg/kg) noticeably reduced the [^{11}C]norepinephrine uptake in the heart (Fig. 1A). This reduction indicated that cocaine blocked the neuronal uptake of [^{11}C]norepinephrine in the sympathetic nerve terminals. This observation is consistent with a recent PET demonstration of the effect of cocaine (2 mg/kg) on (−)-6-[^{18}F]fluoronorepinephrine uptake in the heart.[2]

The present study shows that the uptake of [^{11}C]norepinephrine in the lung was low, and no obvious effect was observed after cocaine pretreatment. There have been conflicting reports on the cocaine-sensitive uptake mechanism of norepinephrine in the lung.[12,13] The present results indicate that such an uptake mechanism of norepinephrine in the lung is unlikely.

In the present study the cocaine congener [^{11}C]β-CIT was used to examine the recognition sites of cocaine.[14] The cardiac uptake of [^{11}C]β-CIT was inhibited by cocaine (5 mg/kg) (Fig. 1B). This result shows that cocaine and [^{11}C]β-CIT bind to the same sites, but Fowler et al. reported that [^{11}C]cocaine uptake in the baboon heart is not inhibited by unlabeled cocaine; they indicated that the rapid association and dissociation of cocaine in the heart prevents the detection of binding to specific sites.[2] The present results show the advantage of [^{11}C]β-CIT over [^{11}C]cocaine in characterizing the cocaine binding site in the heart.

A significantly high concentration of cocaine has been demonstrated in the lungs of those who have died of cocaine overdose,[15] but this observation was not confirmed by Volkow et al.[1] who administered [^{11}C]cocaine to humans but found no remarkable accumulation in the lung. One reason for this discrepancy may be that the affinity of [^{11}C]cocaine is too low to demonstrate specific binding *in vivo*.[2] [^{11}C]β-CIT has a high affinity for monoamine transporters[8,14] and may therefore be a more suitable radioligand for the characterization of the cocaine binding site in the lung. Here the pulmonary uptake of [^{11}C]β-CIT was reduced by 50–65% by pretreatment with cocaine. The difference between the lung and heart in the cocaine effects could be attributed to the difference in nonspecific binding of the two organs. The effects of regional blood flow change after cocaine administration cannot be ruled out; but a 30 min infusion of 0.25 mg/kg/min of cocaine into pigs had no reported effect on myocardial blood flow.[16] The radioactivity in the plasma might decrease rapidly in this experiment, since in a human experiment with [^{11}C]β-CIT, the radioactivity in the plasma was very low.[8] The large amount of serotonin transporter in the lung[17] is thought to be the reason for the pulmonary accumulation, but several accumulation mechanisms have been reported for the pulmonary uptake of xenobiotics.[18]

Although the precise mechanism remains to be clarified, the pulmonary accumulation of cocaine may be an important factor in the pharmacokinetics and prolongation of the toxic effects of cocaine.[19] And [^{11}C]β-CIT is a useful tool to detect the peripheral accumulation of cocaine in cocaine abusers.

The accumulation of [^{11}C]β-CIT in the liver is in agreement with previous studies with [^{123}I]β-CIT.[5-7] High cocaine uptake in the liver has been reported in human postmortem tissue[15] and *in vivo* by PET.[1] The hepatic uptake of [^{11}C]cocaine has been assumed to represent the binding to the high affinity site for cocaine.[1,20] But the finding of an elevated hepatic uptake following pretreatment with cocaine indicates that simply a saturable high affinity binding site for cocaine does not provide the complete explanation. The increase may be a consequence of the displacement of [^{11}C]β-CIT from peripheral and central binding sites.[8]

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