DETERMINATION OF THE CONCENTRATIONS OF D-AMPHETAMINE, NEUROTRANSMITTERS AND VARIOUS METABOLITES IN MICRODIALYSATES TAKEN FROM THE BRAINS OF FREELY-MOVING RATS

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INTRODUCTION

In a previous study, we demonstrated that d-amphetamine-induced increases in striatal dopamine efflux measured by microdialysis in freely-moving rats were correlated with the plasma concentrations of the drug (Rowley et al, 2012, Neuropharmacology 63:1064-74). In this investigation, we have taken this technology one step further by exploring the relationship between d-amphetamine-induced changes in the efflux of dopamine and its metabolites in nucleus accumbens and acetylcholine in the prefrontal cortex and the concentration of d-amphetamine in the same microdialysate sample.

METHODS

Two 2.0 mm microdialysis probes were stereotaxically implanted into the nucleus accumbens (AP +2.2 mm, ML ±1.5 mm, DV -8.0 mm, relative to bregma) and frontal cortex (AP +3.2 mm, ML ±2.5 mm, DV -4.0 mm) of isoflurane-anaesthetised, male, Sprague Dawley rats (~300-350 g). After 16 h recovery, 20 min microdialysate samples (1.2 μl/min artificial CSF [aCSF] for dopamine and metabolites or aCSF+1.0 μM neostigmine for acetylcholine) were taken from freely-moving rats for 3 h following the administration of saline or d-amphetamine (0.5 mg/kg, sc).

Dopamine, and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured by ALEXYS HPLC-EC (Antec, Leiden) and acetylcholine was measured by ALEXYS™ UHPLC-EC (Antec, Leiden).

Maximum dopamine levels: 462±54 fmol/μl

Maximum acetylcholine levels: 462±54 fmol/μl

RESULTS

• d-Amphetamine (0.5 mg/kg, sc) produced rapid increases in dopamine efflux in the nucleus accumbens with a maximal increase at 40 min of 53.8±9.7 fmol/5μl (476% baseline) (Figures 1 and 4).

• Concomitant decreases in DOPAC and HVA with maximum falls to 944±47 fmol/5μl (41% of baseline at 60 min) and 779±29 fmol/5μl (66% of baseline at 80 min), respectively, occurred after administration of d-amphetamine (Figure 3).

• The extracellular concentration of d-amphetamine in the nucleus accumbens reached a maximum of 44.8±12.9 nm/ml at 40 min. The time-course and profile of d-amphetamine levels mirrored the changes in dopamine efflux in this brain region (Figure 4).

• d-Amphetamine produced a more gradual increase in acetylcholine efflux in the prefrontal cortex with a maximal increase at 60 min of 462±54 fmol/10μl (389% baseline) (Figures 2 and 5).

• There was also similarity between the time-course and magnitude of changes in d-amphetamine concentrations and acetylcholine levels in prefrontal cortex microdialysates (Figure 5).

• Comparing extracellular d-amphetamine concentrations in the nucleus accumbens and prefrontal cortex revealed that they were almost identical (Figure 6).

• d-Amphetamine concentrations were highly correlated with both the magnitude of increases in the efflux of dopamine in nucleus accumbens (r=0.967) and acetylcholine in prefrontal cortex (r=0.879) in individual animals (Figures 4 and 5).

CONCLUSIONS

RenaSci and pharm-analyl in collaboration have demonstrated that it is feasible to measure the concentration of d-amphetamine in intracerebral microdialysate samples in addition to neurotransmitters and their metabolites. These microdialysis experiments demonstrate that the actions of d-amphetamine on the efflux of dopamine in the nucleus accumbens and acetylcholine in frontal cortex are highly correlated with the concentration of the drug in the extracellular fluid surrounding the sampling sites.