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Sulpiride, but not SCH23390, modifies cocaine-induced conditioned place preference and expression of tyrosine hydroxylase and elongation factor 1α in zebrafish

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ABSTRACT

Finding genetic polymorphisms and mutations linked to addictive behavior can provide important targets for pharmaceutical and therapeutic interventions. Forward genetic approaches in model organisms such as zebrafish provide a potentially powerful avenue for finding new target genes. In order to validate this use of zebrafish, the molecular nature of its reward system must be characterized. We have previously reported the use of cocaine-induced conditioned place preference (CPP) as a reliable method for screening mutagenized fish for defects in the reward pathway. Here we test if CPP in zebrafish involves the dopaminergic system by co-treating fish with cocaine and dopaminergic antagonists. Sulpiride, a potent D2 receptor (DR2) antagonist, blocked cocaine-induced CPP, while the D1 receptor (DR1) antagonist SCH23390 had no effect. Acute cocaine exposure also induced a rise in the expression of tyrosine hydroxylase (TH), an important enzyme in dopamine synthesis, and a significant decrease in the expression of elongation factor 1α (EF1α), a housekeeping gene that regulates protein synthesis. Cocaine selectively increased the ratio of TH/EF1α in the telencephalon, but not in other brain regions. The cocaine-induced change in TH/EF1α was blocked by co-treatment with sulpiride, but not SCH23390, correlating closely with the action of these drugs on the CPP behavioral response. Immunohistochemical analysis revealed that the drop in EF1α was selective for the dorsal nucleus of the ventral telencephalic area (Vd), a region believed to be the teleost equivalent of the striatum. Examination of TH mRNA and EF1α transcripts suggests that regulation of expression is post-transcriptional, but this requires further examination. These results highlight important similarities and differences between zebrafish and more traditional mammalian model organisms.

1. Introduction

Future strategies for medical treatment of psychological disorders with a strong genetic component, including drug abuse, are likely to include personalized pharmaceutical therapy based on polymorphisms in one or more pathologically related genes. Several heritability studies conducted on monozygotic twins have indicated a strong genetic component for drug abuse consistent with a relatively small number of relevant genes (Kendler and Prescott, 1998; Kendler et al, 2003). However, it is likely that several such polymorphisms lead to the same endpoint, given the complexity by which the brain is affected by addictive drugs (Goldman et al, 2005). Several such polymorphisms have been identified, but finding new targets remains a major goal of addiction research today. Forward genetics in model organisms is a powerful, unbiased approach to finding such novel targets. While at present the use of forward genetics is becoming more popular in rodents (Adams and van der Weyden, 2008), the technique is used much more frequently and extensively in invertebrates and zebrafish. Forward genetics in these organisms has been used to identify novel genes affecting physiology and behavioral sensitivity to addictive drugs (Darland and Dowling, 2001; Ninkovic and Bally-Cuif, 2006; Wolf and Heberlein, 2003).

Addictive drugs have distinct molecular targets and thereby have different physiological effects; yet, by definition, all share the common ability to create a state by which the individual (human or model organism) compulsively seeks the drug in spite of adverse

Abbreviations: CPP, conditioned place preference; DR1, dopamine receptor type 1; DR2, dopamine receptor type 2; Dm, medial zone of the dorsal tegmental area; EF1α, elongation factor 1 alpha; NAc, nucleus accumbens; PPa, anterior preoptic area; PT, posterior tuberculum; TH, tyrosine hydroxylase; Vd, dorsal nucleus of the ventral telencephalic area; VTA, ventral tegmental area; Vv, ventral nucleus of the ventral telencephalic area.

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consequences and to the exclusion of other normally pleasurable stimuli (DSM-IV-TR, 2000). All addictive drugs and naturally pleasurable stimuli also elevate dopamine concentration in the nucleus accumbens (NAc) of the ventral striatum (DiChiara and Imperato, 1988). The NAc and the afferent dopaminergic neurons of the ventral tegmental area (VTA) have long been considered the central pathway governing reward, or reinforcement of behavior (Pierce and Kumaresan, 2006; Wise, 2004). This central pathway also includes distinct but parallel circuits containing other clusters of midbrain dopaminergic neurons and projections to the frontal cortex in mammals (Ikemoto, 2007). Numerous types of experimental paradigms have been developed to measure the rewarding properties of addictive drugs in model organisms. Conditioned place preference (CPP) is a classic experimental paradigm in which the model organism is exposed to a primary stimulus in the context of certain environmental cues (Tzschentke, 1998). The degree of behavioral reinforcement is measured by how frequently the animal approaches the environmental cues in the absence of the primary stimulus in subsequent trials. CPP has proven amenable to the study of reward in most vertebrate model organisms, including zebrafish.

The zebrafish is a popular model organism because it can be readily manipulated genetically, it can be raised in large numbers similar to flies and worms, and it shares many common features of development and the basic neurological layout with mammals. For example, there have been a number of studies indicating that zebrafish have a central dopaminergic pathway analogous to the midbrain–forebrain pathways described in mammals (Rink and Wullimann, 2002, 2004). Furthermore, several studies have documented drug-induced CPP in zebrafish, as well as, abnormal CPP in certain mutagenized families (Darland and Dowling, 2001; Lau et al, 2006; Ninkovic and Bally-Cuif, 2006; Ninkovic et al, 2006; Swain et al, 2004; Webb et al, 2009). In our previous work, we reported three families with abnormal cocaine-induced CPP (Darland and Dowling, 2001). These types of studies validate the potentially powerful approach of applying forward genetic approaches in zebrafish to find novel genes affecting addiction in humans. What remains to be seen is whether the molecular substrates that underlie reward in the zebrafish are analogous to those in mammals.

Cocaine is believed to raise the level of dopamine in the NAc by blocking monoaminergic transporters, principally the dopamine transporter (DAT) (Hall et al, 2004). The rewarding effect is therefore likely mediated through dopamine receptors in the NAc (Holmes et al, 2004). It is not yet known if the same is true for cocaine-induced CPP in zebrafish. Similarly, several studies have examined gene expression changes in the mesolimbic reward pathway after acute and chronic drug exposure to determine the molecular mechanisms underlying the shift in behavior from casual use to compulsion, as well as, genetic vulnerability to drug abuse (Goldman et al, 2005). There has thus been a great deal published about the expression of certain genes after exposure in mammalian systems (Nestler, 2004). While some studies have shown similar drug-induced gene expression changes in zebrafish, these studies did not involve cocaine and often made use of microarrays rather than detailed examination of specific candidate genes (Kily et al, 2008; Webb et al, 2009). Regional changes, statistical stringency and the absence of protein verification may limit the comparison of zebrafish with similar studies in mammals. Examination of genes regulated by addictive drugs in mammals needs to be performed in zebrafish in order to assess similarities and thus validate the model.

The list of genes regulated by addictive drugs is extensive (Nestler, 2004; Nestler, 2005). Among the most studied is tyrosine hydroxylase (TH), an important enzyme in dopamine synthesis. Several studies have reported changes in the expression of this gene after acute and chronic drug exposure, revealing an extensive array of regulatory mechanisms (Beitner-Johnson, 1991; Jedynak et al, 2002; Kumar and Vrana, 1996). The expression changes of genes important in dopaminergic function have to be compared to that of genes normally considered unaffected by the same stimuli. Among these so-called house-keeping genes, elongation factor 1α (EF1α) a protein involved in translational elongation (Negrutskii and El'skaya, 1998), has emerged as one of the more consistent normalizing controls, at least in terms of transcription analysis (Tang et al., 2007). Frequently, however, regulation of the control genes turns out to be more complex and profound than that of genes considered directly relevant to the process in question. In the current study, the regulation of TH and EF1α to a single cocaine exposure with and without dopaminergic antagonists was examined in zebrafish for comparison with what has been reported in mammalian species.

2. Materials and methods

2.1. Fish maintenance and husbandry

Fish were maintained at the University of North Dakota zebrafish facility in accordance with well-established procedures (Westerfield, 2007). The fish were kept at 28.5 °C on an aquatic habitat freestanding system with a 14–10 light–dark cycle. Water conditions were kept at a pH of approximately 7.8 and conductivity typically between 800 and 1000 μS. Fish were fed twice daily with artemia and pelleted food. AB strain zebrafish (Harvard Biological Laboratories) hatched and raised through four generations in North Dakota were used for this study (IACUC number 0606-1). All studies involved male sibling fish from single clutches 6–8 months old. In previous experiments males were found to be more consistent in their behavioral responses. However, since the completion of this study, we have found similar results with both sexes (data not shown).

2.2. Conditioned place preference

CPP was performed similarly to what has been previously described (Darland and Dowling, 2001), with some notable modifications. The CPP chamber was adapted from that used previously to include three compartments with removable barriers (Fig. 1A). Fig. 1A shows artificial plants in the front section that were not used in the CPP experiments, but provide contrast in the picture. Visual cues included duct tape that was wrapped around the rear of the apparatus, while the front remained clear. Fish also had the rear laboratory wall and differential light shading to use as visual cues. The apparatus had walls dividing it into three equal runways containing 1 L of water each. The runways were completely isolated from one another: there was absolutely no chance of leakage between them. Removable barriers compartmentalized each runway into three sections. The middle section contained twice the volume of the end compartments, which were equal in volume. While the barriers between chambers within a single runway allowed some exchange, studies with phenol red showed that with a solid barrier in place, this exchange was extremely slow, noticeably slower than the 45-minute time course of the drug exposures described below. Three fish were tested simultaneously in the same apparatus, each in its own runway.

On the first day, fish were introduced to the central compartment of the apparatus and habituated during a one-hour long session in which barriers with holes allowed free access to all three chambers. On days 2 and 3 fish were conditioned without drug by isolation first in the front compartment for 45 min and then in the rear compartment for 45 min. Isolation was accomplished by replacing the passable barrier connecting the end compartment to the middle compartment with a solid barrier. Baseline preference for the rear compartment was determined during a ten-minute test swim the following morning on days 3 and 4. Treatment groups were assembled such that they shared the same average baseline preference for the rear compartment. Since observations were made visually, we do not have data on the overall activity. However, we were able to assess the number of times fish changed compartments during baseline trials to