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ROLE OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN CARDIOVASCULAR CHANGES FOLLOWING CHRONIC TREATMENT WITH COCAINE AND TESTOSTERONE: A ROLE BEYOND DRUG SEEKING IN ADDICTION?

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Abstract—Neural plasticity has been observed in the bed nucleus of the stria terminalis (BNST) following exposure to both cocaine and androgenic–anabolic steroids. Here we investigated the involvement of the BNST on changes in cardiovascular function and baroreflex activity following either single or combined administration of cocaine and testosterone for 10 consecutive days in rats. Single administration of testosterone increased values of arterial pressure, evoked rest bradycardia and reduced baroreflex-mediated bradycardia. These effects of testosterone were not affected by BNST inactivation caused by local bilateral microinjections of the nonselective synaptic blocker $CoCl_2$. The single administration of cocaine as well as the combined treatment with testosterone and cocaine increased both bradycardiac and tachycardiac responses of the baroreflex. Cocaine-evoked baroreflex changes were totally reversed after BNST inactivation. However, BNST inhibition in animals subjected to combined treatment with cocaine and testosterone reversed only the increase in reflex tachycardia, whereas facilitation of reflex bradycardia was not affected by local BNST treatment with $CoCl_2$. In conclusion, the present study provides the first direct evidence that the BNST play a role in cardiovascular changes associated with drug abuse. Our findings suggest that alterations in cardiovascular function following subchronic exposure to cocaine are mediated by neural plasticity in the BNST. The single treatment with cocaine and the combined administration of testosterone and cocaine had similar effects on baroreflex activity, however the association with testosterone inhibited cocaine-induced changes in the BNST control of reflex bradycardia. Testosterone-induced cardiovascular changes seem to be independent of the BNST.

Key words: addiction, steroids, cocaine, baroreflex, BNST, extended amygdala.

INTRODUCTION

Emerging data suggest that abuse of androgenic–anabolic steroids (AAS) is frequently followed by the use of other psychotropic drugs (Arvary and Pope, 2000). It has been reported that cocaine is the drug most frequently coabused by AAS users (DuRant et al., 1993, 1995). In fact, epidemiological and clinical results indicate that AAS users are likely to display higher cocaine intake than non-users (McCabe et al., 2007; Kanayama et al., 2009).

The widespread abuse of cocaine and AAS has stimulated the interest in the study of the toxic effects of these substances (Maraj et al., 2010; van Amsterdam et al., 2010). Accumulating evidence suggests that the abuse of cocaine and AAS is associated with cardiovascular complications (Kloner et al., 1992; Sullivan et al., 1998). Cocaine use induces both acute and chronic cardiovascular effects (Kloner et al., 1992). The acute effects of cocaine are well described and include hypertension, coronary vasoconstriction and cardiac arrhythmias (Kloner et al., 1992; Maraj et al., 2010). Less information is available about the effects of chronic cocaine abuse, but studies have reported cardiomyopathies and myocarditis, arrhythmias, and changes in baroreflex activity following long-term cocaine exposure (Kloner et al., 1992; Engi et al., 2012; Maraj et al., 2010). Unlike cocaine, AAS evokes minor acute cardiovascular side effects (van Amsterdam et al., 2010). However, chronic AAS abuse has been associated with hypertension and cardiac pathologies (Sullivan et al., 1998; van Amsterdam et al., 2010). Importantly, studies in animals suggest that AAS and cocaine are capable of mutually potentiating the cardiovascular effects of each other (Phillis et al., 2000;

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Abbreviations: AAS, androgenic-anabolic steroids; ANOVA, analysis of variance; BNST, bed nucleus of the stria terminalis; coc, cocaine; HR, heart rate; MAP, mean arterial pressure; T, testosterone; veh, vehicle.

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Togna et al., 2003). Indeed, we have recently reported that administration of either testosterone or cocaine for 10 consecutive days in rats produced a range of cardiovascular effects (e.g., mild hypertension, changes in baroreflex activity and stress-evoked cardiovascular responses, and arrhythmias), which were more pronounced when the substances were coadministrated (Cruz et al., 2012; Engi et al., 2012). Although several reports have shown the occurrence of cardiovascular diseases following either single or combined abuse of cocaine and AAS, the mechanisms involved in the physiopathology of these complications are not entirely understood.

Studies using animal models have shown that repeated exposure to cocaine produces morphological and functional changes throughout the brain (Belej et al., 1996; Beveridge et al., 2004; Macey et al., 2004). Although the overall goal of the majority of these studies has been to evaluate neural changes in brain areas that are considered to be components of the neurocircuitry of addiction, significant changes have also been reported in brain regions that regulate cardiovascular function. Indeed, functional changes in the activity of forebrain and brainstem autonomic regions occur even at the initial stages of cocaine exposure and these alterations progress with the prolonged cocaine contact (Beveridge et al., 2004; Macey et al., 2004). Some results have suggested that long-term AAS treatment may also change the activity of brain regions controlling cardiovascular functions (Rosa et al., 2005). These results support the hypothesis that cardiovascular complications associated with chronic exposure to cocaine and AAS may result from neural plasticity in central nervous system regions controlling the autonomic activity. To our knowledge, however, no studies have directly assessed the brain regions involved in cardiovascular complications following cocaine and AAS exposure.

The bed nucleus of the stria terminalis (BNST) is localized in the rostral prosencephalon and is involved in the control of cardiovascular functions (Gelsema et al., 1993; Crestani et al., 2013). Previous studies have reported that either stimulation or inhibition of the BNST evokes changes on blood pressure, heart rate (HR), baroreflex activity and cardiovascular adjustments to physiological challenges (e.g., emotional stress and physical exercise) (Ciriello and Janssen, 1993; Crestani et al., 2008, 2009; Alves et al., 2011). Morphological and functional changes have been observed in the BNST following repeated administration of cocaine and AAS (Belej et al., 1996; DeLeon et al., 2002; Beveridge et al., 2004; Macey et al., 2004; Costine et al., 2010). These results support data suggesting a role of the BNST in reward-seeking, addiction, drug relapse and behavioral changes associated with these substances (Epping-Jordan et al., 1998; Erb and Stewart, 1999; Aston-Jones et al., 2010; Costine et al., 2010; Koob and Volkow, 2010). However, a possible involvement of the BNST in cardiovascular toxicity associated with drug abuse has never been investigated. Therefore, the goal of the present study was to investigate the involvement of the BNST on changes in blood pressure, HR and baroreflex activity following either single or combined administration of cocaine and testosterone for 10 consecutive days in rats.

EXPERIMENTAL PROCEDURES

Animals

Twenty-six male Wistar rats weighing 200 g in the beginning of the experiments were used. Animals were obtained from the animal breeding facility of the Univ. Estadual Paulista – UNESP (Botucatu, SP, Brazil) and were housed in plastic cages in a temperature-controlled room at 24oC in the Animal Facility of the Laboratory of Pharmacology, School of Pharmaceutical Sciences, Univ. Estadual Paulista – UNESP. They were kept under a 12:12-h light–dark cycle (lights on between 6:00 am and 6:00 pm) with free access to water and standard laboratory food. The injections of drugs and the cardiovascular analysis were carried-out during the light phase. Housing conditions and experimental procedures were approved by the Ethics Committee for Use of Animals of the School of Pharmaceutical Science/UNESP.

Treatment

Animals were randomly divided into four groups: (i) vehicle (almond oil, 1 ml/kg, s.c.) + vehicle (0.9% NaCl, 1 ml/kg, i.p.) (veh + veh) (n = 6); (ii) testosterone (10 mg/kg, s.c.) + vehicle (T + veh) (n = 5); (iii) vehicle + cocaine (20 mg/kg, i.p.) (veh + coc) (n = 6); and (iv) testosterone + cocaine (T + coc) (n = 5). Animals received treatments once daily for 10 consecutive days. The doses and treatment regimen were chosen based on our previous studies (Cruz et al., 2012; Engi et al., 2012).

Surgical preparation

On the last day of treatment, and three days before the trial, rats were anesthetized with tribromoethanol (250 mg/kg, i.p.). After scalp anesthesia with 2% lidocaine the skull was exposed and stainless-steel guide cannulas (26G) were bilaterally implanted into the BNST at a position 1 mm above the site of injection, using a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Stereotaxic coordinates for cannula implantation into the BNST were: antero-posterior = +8.6 mm from interaural; lateral = 4.0 mm from the medial suture, ventral = −5.8 mm from the skull with a lateral inclination of 23° (Paxinos and Watson, 1997). Cannulas were fixed to the skull with dental cement and one metal screw. After surgery, the animals received a poly-antibiotic (Pentabiotico®, Fort Dodge, Campinas, SP, Brazil), with streptomycins and penicillins, to prevent infection and the non-steroidal anti-inflammatory flunixine meglumine (Banamine®, Schering Plough, Cotia, SP, Brazil) for post-operation analgesia.