Caffeine enhances and accelerates the expression of sensitization induced by coca paste indicating its relevance as a main adulterant

Caffeine and locomotor sensitization

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Abstract

Background and Objectives: Caffeine is an active adulterant found in several drugs of abuse including coca paste (CP). We had previously demonstrated that caffeine potentiated the acute stimulant effect induced by CP seized samples. The role of caffeine in the expression of sensitization elicited by a CP seized sample (CP1) was here evaluated.

Methods: CP1 (equivalent dose of 10 mg/kg of cocaine), cocaine (pure, 10 mg/kg), a combination of cocaine 10 mg/kg plus caffeine 2.5 mg/kg (CP1-surrogate) and saline (control) were intraperitoneally injected in male rats under two different sensitization schedules. Ambulatory locomotion was recorded in 58 animals.

Results: After 5 daily CP1 injections and 5 days of withdrawal, CP1-challenged animals displayed a more robust sensitization than cocaine-treated animals. When a 3 injections-regime of CP1-surrogate or cocaine was assayed, only CP1-surrogate was able to elicit sensitization.

Discussion and Conclusions: Caffeine enhances and accelerates the CP1-induced sensitization.

Scientific Significance: Results may shed light on the fast and high dependence observed in CP users.

Keywords: Coca paste, Cocaine, Caffeine, Sensitization, Addiction

INTRODUCTION

Coca paste (CP) is an illicit drug of abuse which consumption has increased in recent years. Whilst worldwide crack is the most well-known form of cocaine used for smoking, CP is another smoked cocaine form commonly used in several South America countries[1-3]. CP can be differentiated from crack since CP is one of the earliest intermediate products obtained during the extraction of the alkaloid cocaine from coca leaves (*Erythroxylon coca*)[1]. As a result of this chemical process, cocaine hydrochloride is reached. In contrast, cocaine hydrochloride is used as a precursor for crack preparation[4-6]. CP contains a high, although variable amount of cocaine (base), low proportions of chemical substances such as kerosene, sulfuric acid, benzoic acid and other alkaloids which occur as a natural result of the extraction process[1,7].

Like crack, CP is a low price drug, and although illegal, is readily available. In humans, consumption of CP produces an intense feeling of euphoria combined with psychomotor alterations. Common users of CP quickly develop a high level of dependence with high rates of relapse. Behavioral alterations such as cognitive deficits, increased levels of impulsivity and aggressiveness and sleep disturbances are also seen. These behavioral traits conform a clinical profile shared by all CP users[2,8].

It has been proposed that the propensity to develop addiction to a particular drug depends on how fast the substance reaches the brain, determining the relevance of the route of administration[9,10]. This might be one reason why, for example, crack is thought to be more addictive than sniffed cocaine[9,11]. Although other factors should not be ruled out, it is possible that the

pulmonary inhalation used to consume CP could partially explain the high dependence/high rates of relapse observed in CP users.

We have previously reported that, as it happens with other illicit drugs of abuse[12], CP seized samples contain passive (innocuous) and active adulterants[13]. Among active adulterants, caffeine, a natural xanthinic alkaloid, is one of the most commonly found[13]. In rodents, we have shown that the acute systemic administration of a series of CP seized samples obtained from police drug raid, has a significantly higher stimulant effect compared with purified cocaine (hydrochloride) administered under similar conditions (without adulterants). Interestingly, we determined that those CP seized samples adulterated with a high caffeine proportion induced the higher stimulant effect. We have proposed that the content of caffeine in CP seized samples should be taken into consideration when analyzing the pharmacological effects of CP[13].

Caffeine is a legal substance consumed worldwide to improve attention and overall performance. Extensive use of caffeine may lead to the development of mild psychological and physical dependence[14]. Caffeine is found in many commercial products, but it is also used as an active adulterant in drugs of abuse[6,12,15-17]. It has been reported that street cocaine and methamphetamine contain caffeine not only to increase the volume and/or weight, but probably to enhance the reinforced property as well as the stimulant effect[12,16]. In addition, caffeine can be volatilized[18], which would be one of the reasons why it is found as an adulterant in CP samples. However, little research on the consequences induced by caffeine in CP seized samples and the expression of the clinical profile seen in patients is available.

It is known that an initial exposure to cocaine results in an increased motor activity, which is further enhanced after repeated administration and a following withdrawal period. This phenomenon known as sensitization can produce enduring molecular, cellular and behavioral changes that resemble some addiction-related features seen in humans[19-22] and can predict the ability of the drug to reinstate drug-seeking behavior in rats[21,22]. The process of sensitization has an initiation phase in which animals display a gradual increase in the locomotion following daily cocaine administration. A second phase is characterized by a persistent hyper-responsiveness to the drug after cessation of administration and includes certain neuroadaptative modifications[21-24].

The present work was designed to determine if a selected CP seized sample was able to evoke locomotor sensitization after a repeated administration schedule. As caffeine was the only active adulterant found in this CP seized sample[13], its role in the rat's locomotor sensitization was also evaluated.

METHODS

Animals

Wistar male rats (IIBCE animal facilities, Montevideo) weighing 250-310 g were employed. All animals were housed in groups of 5 in plastic cages (50 cm × 37.5 cm × 21 cm) and kept under controlled conditions (temperature 22 ± 2°C, 12-h day-night cycle, lights on at 7:00 am) with food and water available *ad*

libitum. All procedures were approved by the IIBCE Bioethics Committee's requirements and carried out under the current national ethical regulations.

Drugs

CP was supplied by the Technical Forensic Institute (Uruguay) from a seized drug shipment targeted for the Uruguayan illegal drug market, with the authorization of the National Drugs Board and the Ministry of Public Health (Uruguay). In the present study, CP1 was selected among a group of CP seized samples. Cocaine hydrochloride (pure) was generously donated by Laboratory Verardo & Cia (Argentina) and caffeine was obtained from Sigma Aldrich (Germany). CP1 was dissolved in a vehicle solution containing 2 % hydrochloric acid (HCl) and enough sodium hydroxide to titrate the solution to a pH of= 6.3. Cocaine hydrochloride and caffeine were dissolved in saline. A previous chemical analysis indicated that CP1 contained 68.9 % of cocaine base and 15.0 % of caffeine. No other adulterants were found in CP1 and impurities were present in a very low proportion[13]. We have previously published data regarding the acute stimulant and neurochemical effect induced by this CP sample[13].

Experimental groups

CP1 was injected at an equivalent cocaine dose of 10 mg/kg (final dose 13.0 mg/kg) and cocaine was administered at 10 and 5 mg/kg. Caffeine was administered alone at a dose of 2.5 mg/kg and the same dose (2.5 mg/kg) in combination with cocaine 10 or 5 mg/kg, respectively. Control animals were

injected with the corresponding vehicles. The volume of injection was set at 1 ml/kg and corrected when equivalent doses were administered. Caffeine dose was calculated based on the percentage of caffeine content of CP1[13]. A previous study showed that combination of cocaine and caffeine at doses equivalent to their content in CP1 exerted the same stimulant effect and that can be used as a valid surrogate to study its effects[13]. Here, we used the combination of cocaine (10 mg/kg) and caffeine (2.5 mg/kg) as a CP1-surrogate.

Behavioral assays

Measurement of locomotor sensitization was carried out in an Open Field (OF) paradigm (a square box of 60 × 60 cm with red 40-cm-high acrylic sides) in a quiet experimental room with controlled temperature (22 ± 2°C). Locomotor activity was recorded automatically by a camera connected to a computer equipped with the Ethovision XT 7.0 software (Noldus, the Netherlands). Using this video tracking software we specifically measured the horizontal locomotor activity defined as the total distance moved in meters (m).

To study the sensitization induced by CP1 and cocaine the following experimental protocol was applied: one day preceding drug treatment, animals were habituated to the OF over a 60 min period (day 0) in which basal locomotor activity was recorded. Later, animals were randomly assigned to different experimental groups which received CP1, cocaine and their respective vehicles daily for 5 days (Protocol I). On day 1 and 5, before the drug or vehicle injection, animals were allowed a 20 min habituation period in the OF prior drug

injection. Locomotor activity was assessed each day for 30 min starting 5 min after the drug or vehicle administration. After the last treatment, rats were kept in their home cages for a 5 days withdrawal period. On the eleventh day, animals were put in the OF for 20 min (habituation prior the drugs injection) and then received a challenge injection according the rat pretreatment. Locomotor activity was recorded for 30 min starting 5 min after the drug or vehicle administration (Fig. 1A shows the experimental schedule).

To study the role of caffeine in the expression of the CP1-induced sensitization a surrogate of CP1 was administered. The following experimental protocol was applied: one day preceding drug treatment animals were habituated to the OF over a 60 min period (day 0) in which basal locomotor activity was recorded. Later, animals were randomly assigned to the different experimental animal groups which received cocaine (10), caffeine (2.5 mg/kg) and a combination of cocaine and caffeine i.e. $Coc_{(10)}$ + $Caff_{(2.5)}$ (as a surrogate of CP1 at an equivalent cocaine dose of 10 mg/kg] and their respective vehicles daily for 3 days (Protocol II). Only on day 1 animals were allowed a 20 min habituation period in the OF prior drug injection. Locomotor activity was recorded each day for 60 min starting 5 min after the drug or vehicle administration. After the last treatment, the rats were kept in their home cages for a 5 days withdrawal period. On the ninth day, animals were put in the OF for 20 min (habituation prior the drugs injection) and then received a challenge injection according the rat pretreatment. Locomotor activity was registered for 60 min, 5 min after the drug or vehicle administration (Fig. 1B shows the experimental schedule).

During all experiments the OF was cleaned with alcohol 30 % before placing the following rat. All experiments were done between 9 a.m. and 3 p.m.

Statistical analysis

Data are given as Mean ± Standard Error of the Mean (SEM) and were analyzed by two-way (time and pretreatment) analysis of variance (ANOVA) for repeated measures followed by post hoc Newman-Keuls multiple comparison test and by one-way ANOVA for independent measures (treatment) followed by Newman-Keuls test. Statistical significance was set at $P<0.05$.

RESULTS

CP1- and cocaine-treated animals developed and expressed sensitization

Figure 2 A and B show the effect of CP1 and cocaine treatment in locomotor activity during 5 days and on challenge day. In Fig. 2A, two-way ANOVA revealed a significant effect of the treatment [$F_{(3,15)}= 35.92$, $P < 0.0001$] but not for time [$F_{(4,60)}= 2.05$, $P = 0.09$] or the treatment x time interaction [$F_{(12,60)}= 1.37$, $P = 0.20$]. Results of post-hoc Newman-Keuls suggest that a development of sensitization was achieved after the repeated injection of CP1 and cocaine since a gradual rise in the distance moved was observed in both groups in comparison with their respective controls (Fig. 2A), although some differences were observed. In the case of CP1-treated animals the increase in the locomotor activity reached statistical significance from the second to the fifth

day, whereas in cocaine-pretreated animals the effect was statistically significant only on the last day of the treatment. Additionally, a maximum difference between drugs was evidenced on the third day (Fig. 2A). In Fig. 2B, one-way ANOVA followed by Newman-Keuls revealed that both CP1- and cocaine-treated animals ($P < 0.01$ and $P < 0.05$, respectively) were able to express the sensitization phenomenon. However, a significant difference between CP1 and cocaine ($P < 0.01$) could be observed. The effect of the CP1 challenge on the locomotor activity of the CP1-pretreated animals was significantly higher than that induced by cocaine injection in cocaine-pretreated animals (Fig. 2B). There were no statistical differences during the initiation or expression phase in vehicle-treated animals and challenged with both drugs (Fig. 2 A and B).

Caffeine accelerated and enhanced the sensitization induced by cocaine

In order to confirm whether the presence of caffeine in CP1 could accelerate and enhance the development and expression of sensitization, another group of animals was treated with a combination of cocaine and caffeine at doses equivalent to their content in CP1 (i.e. CP1-surrogate) and the effect of its repeated administration on animal locomotion was compared with that induced by cocaine alone (Fig. 3 A and B). In Fig. 3A, two-way ANOVA revealed a significant effect of the treatment [$F_{(3,14)}= 13.31$, $P < 0.001$] but not for time [$F_{(2,28)}= 3.10$, $P = 0.06$] or the treatment x time interaction [$F_{(6,28)}= 1.19$, $P = 0.33$]. Post-hoc Newman-Keuls showed that the repeated injection of CP1 surrogate or cocaine was able to induced a gradual and significant increase in

the distance moved along the days in comparison with their respective control groups (Fig. 3A), demonstrating a development of sensitization. A maximum difference between drugs was evidenced on the second day ($P < 0.01$; Fig. 2A). In Fig. 3B, one-way ANOVA followed by Newman-Keuls revealed that while cocaine-treated animals seemed to develop sensitization (Fig. 3A) these animals did not express sensitization when challenged with cocaine (Fig. 3B). In contrast, a significant effect on the locomotor activity of CP1-surrogate-treated animals was observed compared with the control group ($P < 0.05$). Moreover, CP1-surrogate induced a more robust effect on animal locomotion compared with cocaine-challenged animals, demonstrating a great influence of caffeine in the expression of the sensitization elicited by CP1 surrogate (Fig. 3B). There were no statistical differences during the initiation or expression phase in saline-treated animals challenged with cocaine or CP1 surrogate (Fig. 3 and B). Moreover, sensitization was not observed in animals pre-treated with caffeine (2.5 mg/kg) or vehicle and challenged with caffeine (2.5 mg/kg; data not shown).

Accordingly with the finding obtained above and in order to continue studying the influence of caffeine in the development and expression of sensitization induced by cocaine (and indirectly by CP1), another group of animals was treated with a combination of a lower dose of cocaine (5 mg/kg) and the same dose of caffeine (2.5 mg/kg). In Fig. 4A, two-way ANOVA revealed a significant effect of the treatment [$F_{(3,17)}= 31.49$, $P < 0.0001$] but not for time [$F_{(2,34)}= 2.88$, $P = 0.07$] or the treatment x time interaction [$F_{(6,34)}= 1.40$, $P = 0.24$]. Post-hoc Newman-Keuls showed that only those animals treated with cocaine (5 mg/kg) plus caffeine (2.5 mg/kg) were able to developed

sensitization. A gradual increase in distance moved was observed in animals treated with cocaine (5 mg/kg) plus caffeine (2.5 mg/kg) compared with the corresponding control group. Cocaine (5 mg/kg) did not modify the animal locomotion along days (Fig. 4A). In Fig. 4B, one-way ANOVA followed by Newman-Keuls revealed that only those animals which were pretreated with the combination of cocaine (5 mg/kg) plus caffeine (2.5 mg/kg) unchained the expression of sensitization (Fig. 4B). A significant increase in distance moved was observed in cocaine (5 mg/kg) plus caffeine (2.5 mg/kg)-treated animals compared with control group ($P < 0.01$) and also with cocaine alone ($P < 0.001$) indicating that caffeine seemed to facilitate the expression of the sensitization even in rats treated with a minor dose of cocaine. There were no statistical differences during the initiation or expression phase in vehicle-treated animals or challenged with cocaine or cocaine plus caffeine (Fig. 4 A and B).

DISCUSSION

The present study demonstrated that rats repeatedly treated with CP1 displays a more robust behavioral sensitization than cocaine-treated animals suggesting an additive action of its main components, cocaine and caffeine. These observations agree with our previous report in which acute administration of a combination of cocaine and caffeine, reaching specific proportions in CP1, acted in an additive way, explaining the potent acute stimulant effect observed[13]. Here, we also demonstrated that the sensitization phenomenon is elicited even in animals treated with the CP1-surrogate during 3 days. Both are important findings since they suggest that caffeine, as an active

adulterant in CP seized samples, could accelerate and enhance the neuroadaptations involved in behavioral sensitization. **Considering that** the occurrence of behavioral sensitization may predict the ability of a drug to induce reinstatement of drug-seeking behavior[21,22], our results would add valuable information about the clinical **outcome** of CP consumers **and the** mechanisms **underlying the high dependence observed in CP users**.

Other studies have already demonstrated interaction between cocaine and caffeine[23-25], although other paradigms were used and a pre-exposure of caffeine, even at higher doses than that used in our work, were assayed. Contrastingly, our results show the relevance of caffeine, even at a very low dose, in the induction of CP1 sensitization using a combined solution with cocaine (mimicking CP1).

Interestingly, during the development of CP1 sensitization, animals showed a progressive increase in locomotor activity until the third day. Then, a steady decrease was observed until the level of the distance moved of cocaine pre-treated animals was reached. This is probably due to the development of caffeine tolerance as previously described[26]. However, this did not abolish the expression of sensitization seen on the challenge (11^{th}) day.

Molecular events underlying the additive action between cocaine and caffeine are not elucidated here although it is most likely to be mediated through a modulation of the dopaminergic (DAergic) and adenosinergic neurotransmission. Several evidences indicate that sensitization induced by cocaine involves modifications in DA transmission[27]. Cocaine blocks DA reuptake increasing DA neurotransmission in different brain regions, especially those involved in motor control and reward[22]. Specifically, the mesolimbic

DAergic system projecting from the ventral tegmental area to the nucleus accumenbs (NAcc) is believed to be related with the neural mechanism of sensitization, as well as the rewarding and addictive properties of cocaine[28]. On the other hand, caffeine is an unspecific antagonist of adenosine receptors, although it exerts its behavioral actions mainly through A1 and A2A receptors[29]. It is well-known that both A1 and A2A receptors co-localize with DA receptors in the NAcc medium spiny neurons influencing their activity[29,30]. Although the relative contribution of A1 and A2A receptors on the locomotor stimulant effect induced by caffeine has not yet been completely elucidated, some evidences suggest a predominant role of A2A receptors, especially at low caffeine doses[31]. Expression of A2A receptors is enriched in NAcc and dorsal striatum, while A1 receptors are widely distributed through the brain but only moderately expressed in those areas[30]. Additionally, it was demonstrated that the specific A2A antagonist SCH58261 but not the A1 antagonist DPCPX increased the locomotor activity in rats **to** a similar extent than caffeine[32]. A2A receptor has also been associated with the regulation of cocaine-induced behavioral sensitization[33]. Accordingly, our findings agree with the evidences published by Filip and collaborators who have already postulated a putative mechanism to explain the enhancement of the expression of cocaine sensitization through an interaction between adenosine A2 receptors and D2 receptors[33]. Further studies should be done to measure the induction of intracellular proteins (e.g. DARPP-32) or changes in protein expression of membrane receptors (A1 and A2 or D1 and D2 receptors) under the experimental protocols used in the present paper.

When **cocaine** is smoked **effects immediately appear suggesting that the drug** is taken up into the brain more rapidly than **intranasal** cocaine[11].

Although the biological and psychological effects of smoked and **intranasal** cocaine are similar, crack use has been associated with increased abuse[9,11] and the same is considered for CP consumption. Here, we demonstrated that caffeine content present in CP seized samples could potentiate the pharmacological effects induced by CP samples adulterated with caffeine. It might be suggested that under a fast route of administration (pulmonary inhalation) the use of CP seized samples containing caffeine (or other active adulterants) produces a more robust psychostimulant effect and increases the likelihood of craving, relapse and dependence. Accordingly, it has been **hypothesized that the rapid delivery of a drug to the brain facilitates its capacity to induce** ~~forms of~~ neurobehavioral plasticity**,** **leading** to a greater incentive motivation for **the drug** (i.e. sensitization) **and** contributing to **its compulsive use**[10]. Moreover, it has been reported that the production process of freebase cocaine and crack eliminated sugar and sugar alcohols (as volume adulterants) but all other cutting substances detected (e.g. caffeine, phenacetine, lidocaine, levamisol) were present in the cocaine base preparations since they were found in the smoke condensates in sufficient amounts[34]. This fact suggests that all these substances, especially caffeine, could reach the brain in CP consumers contributing to the addictive potential of CP.

Adulteration involves the intentional addition of pharmacologically active substances in an attempt to use less of the intended product without making the user aware. In agreement with this issue, our present results demonstrate that, although the amount of cocaine was lesser (5 mg/kg) the sensitization phenomenon also appears as the presence of caffeine (2.5 mg/kg) would

compensate this decrement, boosting equally the effect of cocaine. It is extremely interesting to know that recently the percentage of caffeine found in some CP seized samples is higher than the percentage of cocaine (unpublished data), indicating once again the relevance of adulterants in drugs of abuse.

In this study it was not determined if caffeine in CP seized samples really enhances the motivational value of the drug. Further experiments should be performed using specific paradigms such self-administration, to answer this question.

In conclusion, we demonstrated that the systemic and repeated administration of a CP seized sample elicited a sensitization phenomenon in rats. Our findings indicate also that the presence of caffeine substantially contribute to the development and elicitation of this phenomenon. Through this work we provide useful information about the factors implied in the pharmacological effect of CP. We also highlight the role of active adulterants commonly used in other illicit psychostimulants. Finally, our results agree with proposed mechanisms involving adenosinergic agonism for new treatment aimed to cocaine or CP addiction[35].

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