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Dopamine on D2-like receptors is involved in reward evaluation in water-deprived rats licking for NaCl and water

Maria Elena Canu [1], Davide Carta [1], Emanuele Murgia [1], Gino Serra, Paolo S. D'Aquila *

Dipartimento di Scienze del Farmaco, Università di Sassari, Sassari, Italy

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ABSTRACT

The analysis of licking microstructure provides measures, size and number of licking bouts, which might reveal, respectively, reward evaluation and behavioural activation. Based on the ability of the dopamine D2-like receptor antagonist raclopride to reduce bout size and to induce an "extinction mimicry effect" on bout number, we suggested that the level of activation of reward-associated responses is updated, or "reboosted", on the basis of a dopamine D2-like receptor-mediated evaluation process occurring during the consummatory transaction with the reward. Here we investigate the effects of the dopamine D2-like receptor antagonist raclopride (0, 25, 125, and 250 μg/kg) on the microstructure of licking for water and sodium chloride solutions (0.075 M, 0.15 M, and 0.3 M) in 12 h water-deprived rats. In each session, rats were exposed to brief contact tests (1 min) for each solution. Bout size, but not bout number, was decreased at the highest NaCl concentration. Raclopride reduced lick number owing to reduced bout size, while bout number was either not affected or even increased depending on the dose. These results are in agreement with the previous observations on sucrose licking, and suggest the involvement of dopamine D2-like receptors in an evaluation process occurring during the consummatory transaction with the reward.

1. Introduction

The mesolimbic dopamine system is involved in the ingestion of NaCl solutions. Indeed, in Na-depleted rats the intake of hypertonic NaCl solutions is accompanied by an increase in dopamine levels in the nucleus accumbens (Frankmann et al., 1994) and the preference for NaCl is reduced after lesioning of the ventral tegmental area (Shimura et al., 2002). *In situ* hybridization and ligand binding studies suggested an involvement of this system in the increased salt appetite in rats after Na depletion (Lucas et al., 2003) or treatment with the mineralcorticoid deoxycorticosterone acetate (Lucas et al., 2000). Moreover, in thirsty rats, the dopamine D1-like receptor antagonist SCH 23390 reduced the intake of water and hypotonic and isotonic NaCl solutions (Gilbert and Cooper, 1987), while increasing the intake of hypertonic solutions, which, conversely, was reduced by administration of a dopamine D1-like receptor agonist (Gilbert and Cooper, 1989). In the same experimental conditions, the dopamine D2-like receptor antagonist sulpiride increased the intake of water and hypotonic and isotonic NaCl solutions (Gilbert and Cooper, 1987).

Rats ingesting fluids emit licks which cluster in bouts (Davis, 1989). Within the framework of the incentive salience attribution hypothesis on dopamine's role in motivation (Berridge, 2007), the size and the number of bouts have been suggested to represent hedonic impact ("liking") and incentive salience ("wanting"), respectively (Higgs and Cooper, 1998). Indeed, the bout size, along with the initial lick rate, is sensitive to tastant concentration, while bout number and lick rate later in the session are more sensitive to cues other than the direct contact with the reward, such as post-ingestive cues (Smith, 2001). Thus, regardless of the interpretation in terms of "liking" and "wanting", these measures appear to deal, the former with the consummatory transaction with the reward and reward evaluation, the latter with the response to stimuli other than the direct contact with the reward (D'Aquila, 2010).

Dopamine D2-like receptor antagonists reduce the size of licking bouts for sucrose solutions (D'Aquila, 2010; Genn et al., 2003; Liao and Ko, 1995; Schneider et al., 1990), thus resembling sucrose dilution (Schneider et al., 1990). Recently, we have shown that they also produce an extinction mimicry effect on the time course of the number of licking bouts similar to that observed in instrumental responding for different rewards (Wise, 2004), while dopamine D1-like receptor blockade reduces selectively and directly the emission of licking bouts. On this basis, we suggested that the level of activation of the responses to the reward-associated cues depends on dopamine D1-like receptor stimulation, and is updated, or "reboosted", on the basis of a dopamine D2-like receptor-mediated evaluation process occurring during the consummatory transaction with the reward (D'Aquila, 2010).

To our knowledge, the effect of dopamine antagonists on the microstructure of licking for NaCl has never been examined before.

* Corresponding author. Dipartimento di Scienze del Farmaco, Via Muroni, 23/A, 07100 Sassari, Italy. Tel.: +39 079 228730; fax: +39 079 228715.
E-mail address: dsfpaolo@uniss.it (P.S. D'Aquila).
[1] MEC, DC and EM are equal contributors to this article.

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Here we investigate the effects of the dopamine D2-like receptor antagonist raclopride (Köhler et al., 1985) on the microstructure of licking for NaCl solutions at different concentrations and water, in mildly water-deprived rats. We used a brief contact test procedure involving very limited post-ingestive effects (Cooper and Higgs, 2005). We examined a dose range of raclopride which in a previous study (D'Aquila, 2010) did not affect lick efficiency, i.e. the ratio between ingested volume and lick number, so that the lick number can be considered a fairly accurate measure of whole intake.

2. Methods and materials

2.1. Subjects and drugs

Twenty-three male Sprague–Dawley rats (Harlan, Italy) weighing 350–450 g were used as subjects. The animals were housed in groups of two–three *per* cage in controlled environmental conditions (temperature 22–24 °C; humidity 50–60%; light on at 08:00, off at 20:00), with free access to food and water. The present study was carried out in accordance with the Italian law (D.L. 116, 1992), and in accordance with the "Principles of laboratory animal care" (NIH publication no. 80-23, revised 1996).

The dopamine D2-like receptor antagonist raclopride [S(-)-raclopride-L-tartrate] (Sigma, St. Louis, USA) was dissolved in distilled water, and injected subcutaneously in a volume of 1 mg/ml at the doses of 25, 125 and 250 μg/kg. Vehicle treatment consisted in a 1 ml/kg distilled water administration. The time interval between drug/vehicle treatments and experimental testing was 30 min (D'Aquila et al., 2010).

2.2. Procedure

Behavioural testing was carried out using a multistation lick analysis system (Habitest, Coulbourn Instruments, USA) connected to a computer. Rats were individually placed in a Perspex chamber with an opening in the centre of the front wall allowing access to a bottle spout. The recording period started after the first lick (Cooper and Higgs, 2005). The interruptions of a photocell beam by each single tongue movement while licking the spout were recorded, with a temporal resolution to the nearest 50 ms. The raw data were the number of licks, the number of bouts and the time spent in bouts, and were analysed through Graphic State 3.2 software (Coulbourn Instruments, USA). A bout was defined as a series of a minimum of four licks with pauses no longer than 400 ms (see D'Aquila, 2010). The number of licks *per* bout (lick number/bout number) and the intra-bout lick rate (lick/s within bouts) were then calculated.

The experimental sessions were carried out after 12 h water deprivation. The subjects were first familiarised with the test apparatus in training sessions where they had access to an isotonic (0.15 M) NaCl solution in daily 15 min sessions. The first experimental session was carried out after one week of training phase. A repeated measures design was adopted, with each rat tested at every dose, including vehicle treatment, in 4 experimental sessions. During each treatment session the subjects were exposed to three different NaCl solutions (0.075 M, 0.15 M, and 0.3 M) and water, in 4 consecutive 60 s brief contact tests 1 min apart from each other (Cooper and Higgs, 2005). Both the order of the treatment sessions and that of the within session solution exposure were balanced across subjects according to a modified Latin square design. Experimental sessions were performed 72–96 h apart to avoid carry over effects. All experiments were performed between 09:00 and 14:00. The subjects were tested in cohorts of four.

2.3. Data analysis

Statistical analysis was performed with ANOVA, with *treatment* (3–4 levels corresponding to doses) and *salt* concentration (4 levels) as within-group factors. Occasional missing cells due to the failure of some animals to lick in particular time bins were replaced with the mean values for the appropriate treatment conditions. The data relative to number of licks *per* bout and intra-bout lick rate were both subjected to two different analyses, either including or excluding the highest dose data. Post hoc analysis of the main effects was made using a Newman–Keuls multiple comparison test. When a significant interaction between *treatment* and *salt* was revealed, comparisons between the different treatment conditions were performed by *F*-test for contrasts.

3. Results

Raclopride treatment produced a dose-dependent decrease in lick number, with the two highest doses resulting in a 17.7% and 53.2% reduction with respect to vehicle, regardless of NaCl concentration [*treatment* main effect: $F(3, 66) = 35.19$, $P < 10^{-6}$]. Moreover, ANOVA revealed a statistically significant effect of the factor *salt* [$F(3, 66) = 7.03$, $P = 0.0003$], due to a 25% reduction in the number of licks for the 0.3 M NaCl solution compared to water, regardless of treatment [Newman–Keuls test: $P = 0.001$], with no interaction between the two factors [$F(9, 198) = 0.81$, n.s.] (Fig. 1, top left). ANOVA of bout number data (Fig. 1, top right) showed a statistically significant effect of *treatment* [$F(3, 66) = 4.37$, $P = 0.007$], due to a 63% increase in bout number at the dose of 125 μg/kg [Newman–Keuls test: $P = 0.003$]. Moreover, it was demonstrated a significant effect of *salt* [$F(3, 66) = 3.16$, $P = 0.03$], due to a 22.2% reduction in bout number for the 0.3 M concentration compared to water, while the *treatment* × *salt* interaction was not significant [$F(9, 198) = 1.4$, n.s.].

The analysis of the number of licks *per* bout showed a statistically significant main effect of *treatment* [$F(3, 66) = 12.96$; $P = 10^{-6}$], but not of *salt* [$F(3, 66) = 2.08$; n.s.] with a very significant interaction between the two factors [$F(9,198) = 2.68$; $P = 0.005$]. Further analysis (*F*-tests for contrasts) revealed a decrease of this measure for hypertonic NaCl compared to the hypotonic solution. Moreover, raclopride reduced the mean bout size for all the solutions including plain water. However, bout size for water and the hypotonic solution were reduced even at the lowest raclopride dose, while for the hypertonic solution only the administration of the highest dose produced a significant effect, with the threshold of raclopride effect for the isotonic solution being at the mid dose. Finally, after treatment with the highest raclopride dose a statistically significant increase in this measure was observed for the isotonic solution compared to water (Fig. 1, bottom left). It should be noted that the mid raclopride dose (125 μg/kg) produced on average a 62% decrease in the number of licks *per* bout, thus resulting in a net reduction of ingestive behaviour (i.e. lick number) in spite of the increased bout number (see above).

The intra-bout lick rate data analysis failed to reveal any significant effect involving the factor *salt* [main effect: $F(3, 66) = 0.39$, n.s.; *salt* × *treatment*: $F(3,198) = 0.87$, n.s.], while a significant effect of *treatment* [$F(3, 66) = 14.31$; $P = 10^{-6}$] was revealed, due to a very small but highly statistically significant reduction of this parameter at the two highest doses (Fig. 1, bottom right). After treatment with the highest raclopride dose a number of subjects failed to lick (see legend of Fig. 1). Thus, a further analysis excluding this dose was performed for the number of lick *per* bout and the intra-bout lick rate data, yielding very similar results in the general analysis [NLPB: *treatment*, $F(2, 44) = 9.72$, $P = 0.0003$; *salt*, $F(3, 66) = 1.85$, n.s.; *salt* × *treatment*, $F(6, 132) = 2.82$, $P = 0.012$. IBLR: *treatment*, $F(2, 44) = 5.29$, $P = 0.008$; *salt*, $F(3, 66) = 0.59$, n.s.; *salt* × *treatment*, $F(6, 132) = 0.89$, n.s.], and the same approximate *P* values in the comparisons between the different treatment/solution conditions.

4. Discussion

These results show that at the highest salt concentration there is a reduction of lick number, due to a reduction in the size of licking

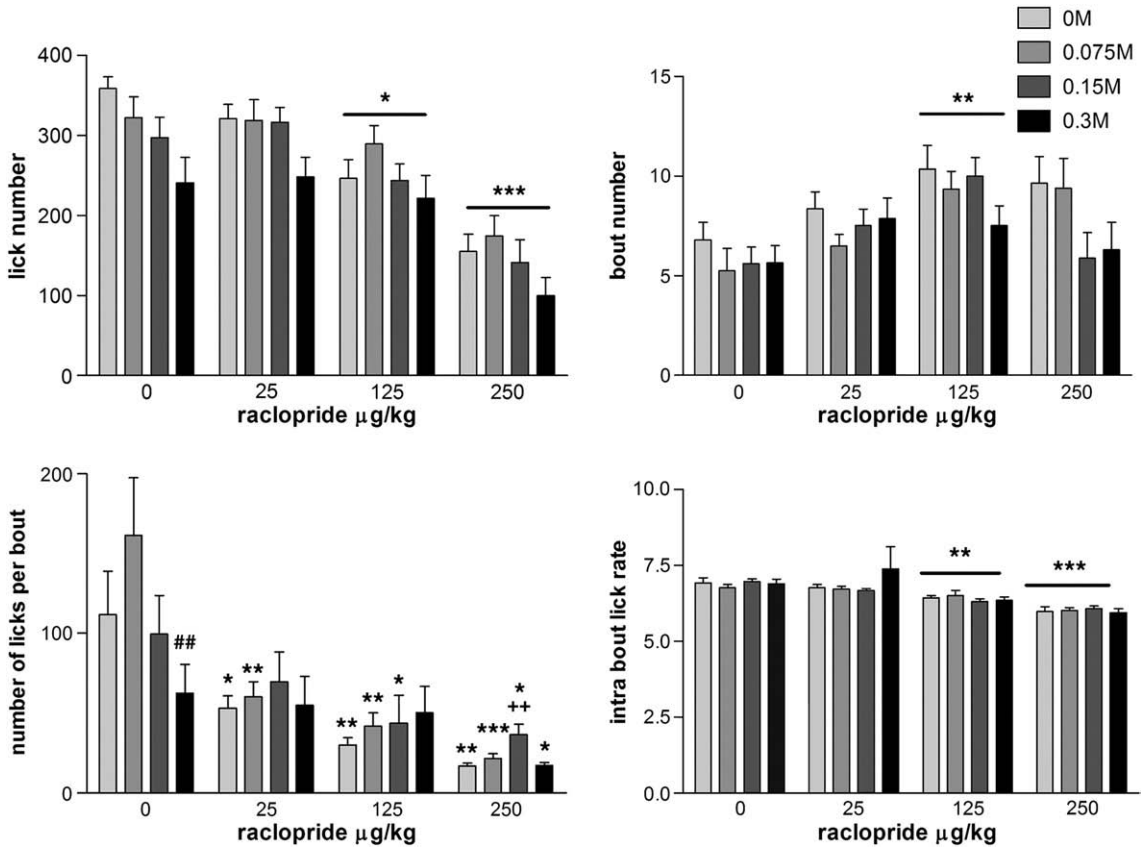

Fig. 1. Effect of the dopamine D2-like receptor antagonist raclopride on the licking microstructure for NaCl. Values represent the mean ± S.E.M. from 23 subjects. The numbers of empty cells due to a subject failing to lick were: vehicle: 2 (0.075 and 0.3 M); low dose: none; mid dose: 2 (0.3 M); high dose (from 0 M to 0.3 M): 1, 2, 8, 6. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$: effect of raclopride; $^{++}P<0.01$: NaCl versus water; $^{\#\#}P<0.01$, with respect to NaCl 0.075 M (ANOVA followed by Newman–Keuls test or *F*-test for contrasts).

bouts. This observation is consistent with the previous studies on the microstructure of licking for NaCl (Baird et al., 2005; Cooper and Higgs, 2005).

Moreover, consistently with earlier observations on sucrose ingestion, the results show the ability of D2-like receptor antagonists to decrease licking through the reduction of the size of bouts, an effect which resembles the effect of reward devaluation (Schneider et al., 1990), along with an increase in their number (D'Aquila, 2010; Genn et al., 2003; Higgs and Cooper, 2000; Liao and Ko, 1995; Schneider et al., 1990), which might resemble the transient increase in instrumental responding observed after either treatment with low doses of neuroleptics or reward devaluation in different experimental paradigms (Wise, 2004). These effects of dopamine D2-like receptor antagonists were interpreted as a reduced "hedonic impact" or "reward evaluation" (see D'Aquila, 2010; Schneider et al., 1990). However, these interpretations appear at variance with the results of taste reactivity studies, which show that not only dopamine antagonists, but even lesioning of dopamine ascending pathways, fail to affect the appetitive fixed action patterns elicited by oral infusion of sucrose, interpreted as signs of "hedonic impact" (Berridge, 2007; Berridge et al., 1989). To reconcile these apparently opposite lines of evidence, we suggested that the taste reactivity studies just reveal a mechanism of detection of the "intrinsic value" of the reward, while the effect of tastant concentration on the size of licking bouts might reveal a further evaluation mechanism, whose task is to determine the "contingent value" of the reward, based on which the level of activation of the reward-directed responses, hence the energetic cost which is congruous for a particular "intrinsic value" reward in a given physiological, psychological and environmental condition, is determined (D'Aquila, 2010). Indeed, nucleus accumbens dopamine depleted rats tend to shift their responses toward the less effortful choices, but still retain the ability to choose the larger reward when no additional effort is required (Salamone et al., 2005, 2007). This account is consistent with the view that dopamine is involved in response effort allocation and cost–benefit based choice (see Salamone et al., 2009). In this study, treatment with the highest raclopride dose resulted in an increased bout size for the isotonic solution compared to water. Within the proposed interpretative framework, one might tentatively speculate that the isotonic solution might represent the reward with the higher "intrinsic value", possibly revealed by the blunting by raclopride of the "contingent" reward evaluation mechanism.

In apparent conflict with these results is the earlier observation that the dopamine D2-like receptor antagonist sulpiride was shown to increase the intake of water, hypotonic and isotonic NaCl solutions in rats after 22 h water deprivation, in a 15 min preference test (Gilbert and Cooper, 1987). However, due to the longer duration of the tests, the observed behaviour was certainly affected by post-ingestive cues, which, according to our suggestion, would elicit activation through a mechanism involving stimulation of dopamine D1-like receptors (D'Aquila, 2010). In keeping with this interpretation, in the cited study, the dopamine D1-like receptor antagonist SCH 23390 reduced water and NaCl hypotonic and isotonic solution intake. However, the differences in the experimental conditions and the use in the cited study of whole fluid intake as a unique dependent measure, prevent any conclusive interpretation.

Neuroleptics were shown to produce specific motor effects on the microstructure of licking, such as increases of the individual lick duration and of the interlick intervals (Gramling et al., 1984; Gramling and Fowler, 1986). These effects are consistent with the reduced intra-bout lick rate observed after treatment with dopamine D2-like receptor antagonists (D'Aquila, 2010; Genn et al., 2003; Higgs and

Cooper, 2000). Consistent results were obtained in the present study: raclopride at the two highest doses reduced the intra-bout lick rate. However, (i) a reduction in bout size was observed also with the lowest dose, which failed to reduce the intra-bout lick rate, and (ii) the effects on bout number were opposite with respect to those on intra-bout lick rate. These observations suggest that the motor effects represented by the reduced intra-bout lick rate after raclopride treatment cannot account for the other microstructural effects.

In conclusion, these observations show that dopamine D2-like receptors play a similar role in the control of the ingestion of sucrose and NaCl solutions, thus providing support to the hypothesis that dopamine on D2-like receptors is involved in the control of responses depending on the direct contact with the reward, such as "contingent" reward evaluation and activation of the consummatory response. This interpretation is consistent with the idea that dopamine D2-like receptors mediate "reboosting" (D'Aquila, 2010), a process whereby the contact with the reward updates the level of incentive salience attribution to reward-associated cues (Berridge, 2007). Moreover, this account fits within the theoretical framework which regards dopamine's role in terms of cost–benefit based choice and response effort allocation (Salamone et al., 2009). However, these results, along with the results from the earlier studies on licking for sucrose, support also the involvement of dopamine D2-like receptors in a process of evaluative perception which might provide a common fundamental element to hedonic experiences of different kinds (D'Aquila, 2010).

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