Dopaminergic correlates of unexpected gains or losses in reward value

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Introduction

Successive negative and positive contrast occurs when the incentive value of a rewarding stimulus is devalued or increased unexpectedly and has been observed in many species ranging from bumble bee to man [1]. In two separate studies, we employed brain dialysis to monitor changes in dopamine (DA) efflux in the Nucleus Accumbens (NAc) of the rat during both negative and positive contrast as evidenced by significant reductions in lick rate when rats were switched unexpectedly from a 32% to 4% sucrose solution, or conversely a significant increase in lick rate following a switch from 4% to 32% relative to controls maintained on a 32% sucrose solution. During successive negative contrast we observed a significant reduction in the magnitude of DA efflux in the NAc [2], whereas positive contrast was accompanied by a significant increase in DA efflux [3].

Successive contrast effects are sensitive to withdrawal from repeated treatment with psychostimulant drugs, which is used as an animal model of depression. Specifically, we have observed both enhanced and prolonged negative contrast [4] and blunted positive contrast effects [5] following withdrawal from escalating doses (1-10 mg/kg) of D-amphetamine. There is also great interest in the possible functional consequences of repeated exposure to the psychostimulant methylphenidate (MPD) treatment during adolescence and preclinical studies report significant changes in mid-brain DA neuronal impulse activity [6]. Given the apparent role for DA in contrast effects, these electrophysiological data encouraged us to examine the effects of repeated exposure to MPD in adolescent rats (post-natal days (PD) 36-42 of age) on successive negative contrast. It is important to note that tests of sucrose downshift occurred on the same days on which DA neuronal impulse activity was measured in MPH treated rats. The possible effect of adolescent treatment with MPD on DA efflux in the NAc evoked by an acute injection of MPD was also examined 10 days after completion of the behavioral experiment.

Methods

Four-week-old male Long–Evans rats (N=40, Charles River, Quebec) were pair-housed, with food and water available *ad libitum*. MPH was prepared daily in 0.9% NaCl solution immediately prior to use and administered at a volume of 1 ml/kg. During PD 36-42, rats were received daily injections of either MPH (2 mg/kg, ip, n=20) or saline (n=20) ~2 pm. Licking sessions were conducted in

Plexiglas chambers (42×38×38 cm). Each lick from the drinking spout delivered a single drop of sucrose solution (0.01 ml). Lick data was collected on a PC using MED-PC software (Med Associates, St. Albans, VT).

Negative contrast. Rats assigned to the MPH and saline treatment groups were further divided into two groups (n=10/group), one presented with a 4% or 32% sucrose solution for a 5-min period once a day in the licking chamber. During PD 45-49 (Shift I), rats given 32% sucrose were switched to a 4% sucrose solution. Following this initial downshift, the 32% groups were shifted back to 32% for 7 days, and then downshifted again to 4% for an additional 5 days (Shift II; PN 57-61). The 4% rats were maintained on the 4% sucrose solution throughout Shift I and II.

Effect of adolescent exposure to MPH on evoked DA efflux in the Nucleus accumbens. Two to three days following the end of Shift II, five rats from each group were implanted unilaterally with stainless-steel guide cannulae (19 gauge x 15 mm) directly over the NAc (from bregma, +1.7 mm anterior and ±1.1 mm lateral; from dura 1 mm ventral). One week following surgery, microdialysis experiments were conducted prior to and following a challenge injection of MPH (2 mg/kg, ip). Samples were collected at 10 min intervals and analyzed immediately for DA using HPLC for 120 min.

Results and Discussion

Control rats treated with saline injections during adolescence (Fig. 1, Sal-tx), had a higher a lick responses for 32% sucrose solution than 4% sucrose (>300 licks/day). Successive negative contrast was clearly evident on PD 45-49, in rats switched from a 32% to a 4% sucrose solution (Shift I). Mean lick rate in the 32%-4% group was significantly lower in comparison to the group maintained on a 4% solution ($p<0.05$). Following the switch back to 32% solution during PD 50-56, lick rate recovered to levels observed prior to Shift I. During Shift II (PD 57-61), a second change from a 32% to a 4% solution again resulted in a significant decrease in lick rate relative to the group maintained on 4% sucrose ($p<0.05$).

Both the initial difference in lick rate in groups treated with 32% as compared to 4% sucrose and successive negative contrast were observed in rats treated with MPH during adolescence (Fig. 1, MPH-tx). During Shift I, MPH-treated rats licked less than saline-treated rats on each of the five days ($P<0.05$). During Shift II, MPH-treated rats switched from the 32% to the 4% solution again showed a decrease in lick rate relative to the group maintained on 4% ($P<0.05$). Notably, on days 58, 60 and 61, the lick rate of MPH-treated rats was significantly elevated relative to saline-controls ($p<0.05$) indicating a significant reduction in negative contrast effect. As this attenuation in the magnitude of negative contrast was observed in rats that were the same age as those displaying a significant reduction in DA neuronal activity [6], it may reflect an important role for the mesocorticolimbic DA system in the manifestation of successive

negative contrast. As such, adolescent exposure to MPD may modulate the motivational consequences of unexpected changes in the value of expected rewards in adulthood.

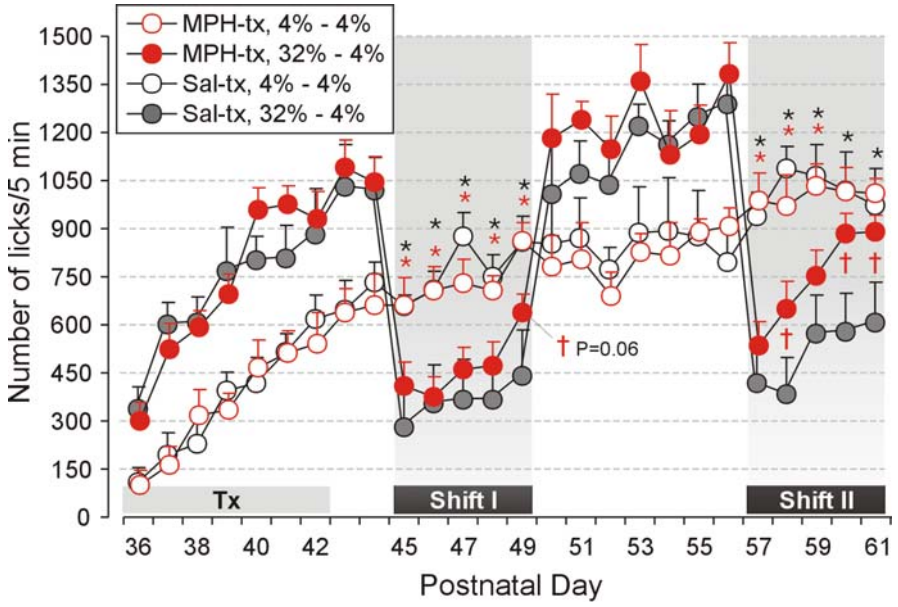

Figure 1. Effects of prior treatment with MPD on successive negative contrast.

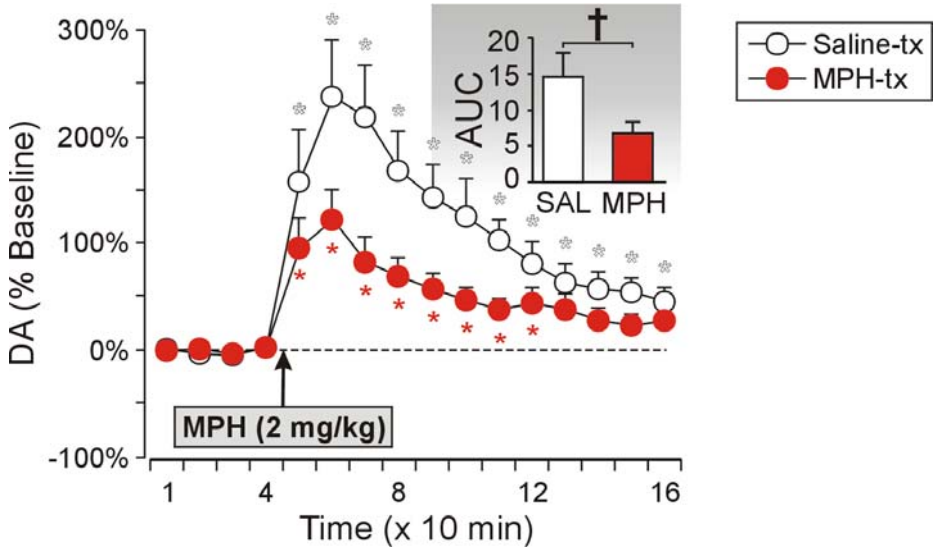

Figure 2. Prior treatment with MPD during adolescence attenuates the magnitude of DA efflux in the NAc evoked by an acute injection of MPD in adult rats.

Effect of adolescent exposure to MPH on evoked DA efflux in the Nucleus accumbens. The basal concentration of DA in the NAc was 1.49±0.16 nM in saline-treated rats, and did not differ significantly from 1.68±0.20 nM in MPH-treated rats (P=0.55). A challenge injection of MPH in adulthood, in rats with adolescent exposure to MPH was followed by an increase in DA efflux in the NA (Fig 3). Importantly, this effect was significantly blunted when compared to saline-control (see *inset*, P=0.044).

These *in vivo* neurochemical data provide further evidence in support of the hypothesis that exposure to MPD in adolescence has long-lasting consequences on the function of brain DA systems. When coupled with the reduction in negative contrast reported above, it may be conjectured that this modulation of brain DA in the NAc and other terminal regions has significant effects on motivation and reward processes.

This research was supported by a grant from the Canadian Institutes of Health Research to AGP.

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5-HT2A receptor antagonism affects encoding of reward-related information in the orbital prefrontal cortex

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Introduction

Serotonin (5-HT) has been suggested to support affective processing (i.e. the ability to evaluate and integrate emotional content) in both human and rodent brain [1,2]. Other experiments in humans and non-human primates show that certain aspects of cognitive flexibility, i.e. reversal learning (sometimes also called affective shifting) depend critically on central 5-HT [3,4]. As the orbital prefrontal cortex is a main site of processing of reward-related information we became interested in answering the question whether 5-HT would be a causal or at least a modulating agent in the coding of reward-related activity in that area. To this end we used the combidrive, which is an array of 12 tetrodes to perform ensemble recordings with a moveable and replaceable microdialysis probe to locally administer pharmacological agents [5]. The combidrive was shown to allow ensemble recordings simultaneously with reverse microdialysis in freely moving rats for periods of up to two weeks, making it suitable for application in behavioural studies. To examine the role of 5-HT in the encoding of orbital PFC reward information we aimed to measure orbital PFC neurons during an operant odour-reversal task in the presence and absence of a selective 5-HT_{2A} receptor antagonist. This receptor subtype was chosen because of its presence in PFC and its role in PFC activity [6,7].

Methods

Data were collected from 5 male Wistar rats. The combidrive and the analysis methods we used for these experiments were previously described [5]. Orbital PFC neurons were measured in an three-way odour discrimination task with 2 different types of reward (Noyes AI and P; previously shown to be differentially preferred [2]) or zero-reward. Over consecutive sessions, the animals were presented with new exemplar odours on morning acquisition trials, which were reversed in afternoon sessions. Measurements were combined with perfusions of a selective 5-HT_{2A} antagonist (M100907 10 µM) or a control solution.

Results and Discussion

During the acquisition sessions, both control and drug groups completed significantly more trials on rewarded odours compared with 'zero-reward'. Latency times were higher for both groups on rewarded vs. non-rewarded trials. Taken together, these data suggest that in both control and drug sessions the animals acquired the different odour-reward contingencies and behaved accordingly. Over a total of 38 recording sessions, 732 neurons were recorded from the orbital PFC (average 19.26 cells/session). Of these neurons, a total of 693 cells were analyzed, yielding 166 (24 %) neurons that were found to show a significant change in firing activity during one or more events in the task. A comparison of all correlates between the drug and the control sessions indicated that there was a significant difference in the number of correlates between rewarded and non-rewarded trials, which was absent when local 5-HT_{2A} receptors were blocked. This preliminary analysis suggests that 5-HT_{2A} receptors are involved in the encoding of orbital PFC reward information.

Supporterd by ZonMW-TOP 912-02-050, Hersenstichting Nederland 14F06.67 and Nederlandse Organisatie voor Wetenschappelijk Onderzoek Grant 903-47-084.

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Motivated behavior, internal bliss and the role of cannabinoid receptors

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Introduction

It has been proposed that endogenous cannabinoids contribute to the valuation of various reinforcers including brain stimulation reward (BSR), highly palatable food and abused drugs, in part by modulating neurotransmitter levels including dopamine [1]. In BSR, rats work vigorously and persistently to trigger electrical stimulation of certain brain regions [2], including the projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), even at the cost of forgoing vital physiological resources. The avidity and persistence of behavior directed at obtaining the electrical stimulation and the agonistic interaction of BSR with natural rewards suggests that the stimulating electrode injects a signal into the brain that mimics the rewarding effect of natural goal objects such as food. Moreover, the potentiation of BSR by abused drugs suggests that the same neurobiological substrates contribute to harmful behaviors as well as to salubrious ones. However, by bypassing much of the input side of these neuronal circuit(s), the rewarding value of BSR is almost immediate. Our prior studies in the NAc showed that coincident changes in firing and subsecond dopamine release occur at cues signaling reward availability during BSR and are functionally linked via D1 receptor activation [3]. Little is known, however, about the precise relationship between accumbal encoding of BSR and endogenous cannabinoid signaling. Here, we combined fast-scan cyclic voltammetry with electrophysiological recordings at the same carbon-fiber electrode in animals trained to press a lever to self-stimulate the VTA [3,4]. Rats were treated with the cannabinoid receptor (CB1) antagonist rimonabant during the session while responding was ongoing. Effects on behavior and neuronal encoding were contrasted between vehicle and rimonabant treatments. The relevance of the data obtained here is discussed in light of the behavioral efficacy of rimonabant in several animal models of reinforcement.

Materials and Methods

Eight adult male Sprague-Dawley rats (300-400 g) were stereotaxically fitted with a guide cannula on the skull above the NAc, a reference electrode (Ag/AgCl) on the contralateral hemisphere and a bipolar stimulating situated above the VTA. Dopamine release was optimized during surgery via fine micromanipulator control of the stimulating electrode. Animals were allowed to recover for at least 48 hr prior to the start of the experiments. During the first day

of experiments rats were tethered by the electrode assembly to a rotating commutator (which allowed for virtually unrestricted movement) and left to habituate to the environment for at least 30 min. Following habituation, a microdrive containing a cylindrical carbon fiber microelectrode pulled in glass capillary (exposed carbon fiber tip; ~ 75-100 μm) was attached to the guide cannula and locked in position. The electrode was then manually lowered to the NAc shell with 0.1 mm precision and then left to stabilize for at least 15 min once the desired location was reached. Rats were trained as previously described [3]. During execution of the behavior, concentration vs. time traces and cyclic voltammograms as well as firing rates were averaged and synchronized with respect to different phases of the task (i.e., lever extension, lever press and stimulation). The CB1 receptor antagonist rimonabant (SR141716A; Research Triangle Institute/NIDA, Durham, NC) was freshly suspended in a 1:1:18 ratio of ethanol, emulphor (Alkamuls EL-620, Rhodia Inc., Cranbury, NJ) and saline (0.9%). Rats performed 30 baseline presses followed by an intravenous bolus dose of vehicle Injected from outside the chamber) and 30 presses and a final bolus of rimonabant (300 μg/kg) and 30 presses, in order to not disturb ongoing behavior. In order to achieve this, Tygon tubing attached to a syringe (1 cc) located outside the chamber will be connected to a fluid swivel and then routed through the electrical commutator (the volume within the tubing from the syringe to the animal will be 600 μl). Following injection of the desired volume, a saline bolus of 1 ml was advanced by the experimenter through the tubing to ensure full intravenous dosing. For intra-cranial infusions, rimonabant was dissolved in pure DMSO, and diluted in aCSF [5]. On the day of the experiment, a dummy cannula was replaced with an injector (Plastics One, Roanoke, VA) coupled to a 1 μl Hamilton micro-syringe via specific diameter connectors and PE tubing coursing through the fluid swivel and the electrical commutator (Crist Instrument, Hagerstown, MD). Animals received the intracerebral injection in the behavioral chamber after the dummy cannulae were removed and the injection cannulae were inserted into the guides. The total volume was injected over 2 min by a programmable syringe pump (Harvard Apparatus, Holliston, MA). After an additional 1 min wait, the injection cannulae were removed, the dummy cannulae were replaced followed by the start of the behavioral session. After the experiment, 0.5 μl of a 2% Chicago Sky Blue solution was microinjected into the VTA immediately before trans-cardial perfusion. Subjects were included in the study if they showed dye covering approximately 70% of the VTA. Changes in dopamine release were assessed using chemometric analysis and principal component regression. Perievent Histograms (PEHs) and rasters depicting time-locked changes in NAc neuronal firing rate were also erected around each event.

Results and Discussion

The present findings suggest that endogenous cannabinoid binding to CB1 receptors facilitates the encoding of goal-directed behavior (see **Figure 1**). The main effect of cannabinoid receptor blockade appears to be a perturbation of cue-

evoked encoding in the NAc, and not a reduction in the reinforcing value of the stimulation. The finding that CB1 receptor blockade sufficient to alter behavioral responding and dopamine output indicates that endogenous cannabinoids are important in the orchestration of normal appetitive behavior as well as disorders of motivation such as addiction.

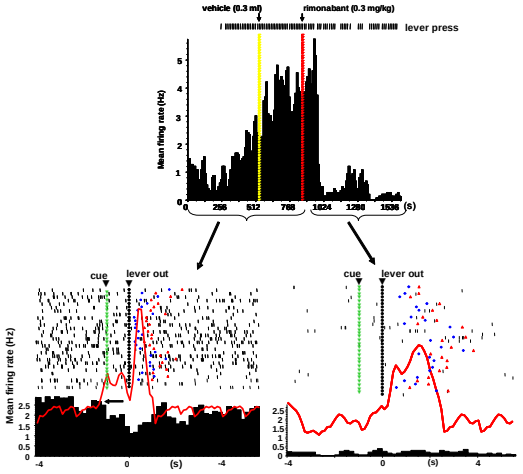

Figure 1. Injection of vehicle during BSR did not affect behavior; while rimonabant administration elicited a profound increase in response latencies (vertical ticks, top panel).The histogram shows that overall firing rate increased following the initiation of behavioral responding, was unaffected by vehicle but was reversed to pre-responding levels by rimonabant (middle panel). A closer examination of the firing patterns revealed that this neuron exhibited inhibitory electrophysiological encoding of cue onset which coincided with the onset of the rise in cue-evoked dopamine release (indicated by the arrow, bottom left). Importantly, these encoding patterns were no longer observed following rimonabant administration (bottom right), and this lack of encoding occurred precisely at the time animals displayed longer latencies to press (top).

This work was supported by a NIDA grant (DA022340 to JFC).

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Bimodal effect of peripherally-administered ghrelin on the mesolimbic dopamine system is dependent on the food consumption

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Introduction

Ghrelin is a powerful orexigenic peptide released from the stomach that targets CNS receptors to induce orexigenic behavior [1,2]. The present study aimed to identify the neuronal interactions that mediate the effects of peripherally-administered ghrelin on mesolimbic dopamine neurons.

Methods

Ghrelin was administered peripherally (3 nmol, i.v.) as well as locally into the ventral tegmental area (VTA) (0.3 nmol/side) to the rats. Dopamine in the nucleus accumbens (NAc) was measured by microdialysis.

Results and Discussion

Peripheral administration of ghrelin inhibited dopamine levels in the NAc when food was deprived following ghrelin administration. This inhibitory effect was mediated through an increase in glutamatergic inputs to the VTA that activate GABA interneurons and subsequent inhibition of dopamine neurons via $GABA_A$ receptors (Fig 1(1)). In contrast, when animals consumed food following ghrelin administration, dopamine levels increased robustly. This stimulatory effect was mediated through an increase in glutamatergic inputs to the VTA that directly activate dopamine neurons via NMDA receptors. Importantly, both the inhibitory.and stimulatory effects of ghrelin required activation of GHSRs in the VTA (Fig 1 (2)). When ghrelin was injected locally into the VTA, dopamine release in the NAc increased regardless of food consumption, supporting the local action of ghrelin on dopamine neurons. In addition, the role of GHSRs in the VTA in the orexigenic effect of peripherally-administered ghrelin was demonstrated. In conclusion, peripherally-administered ghrelin induces bimodal effects on mesolimbic dopamine neurotransmission depending on the execution of food consumption. These findings propose a novel mechanism for ghrelin to regulate appetite and food consumption.

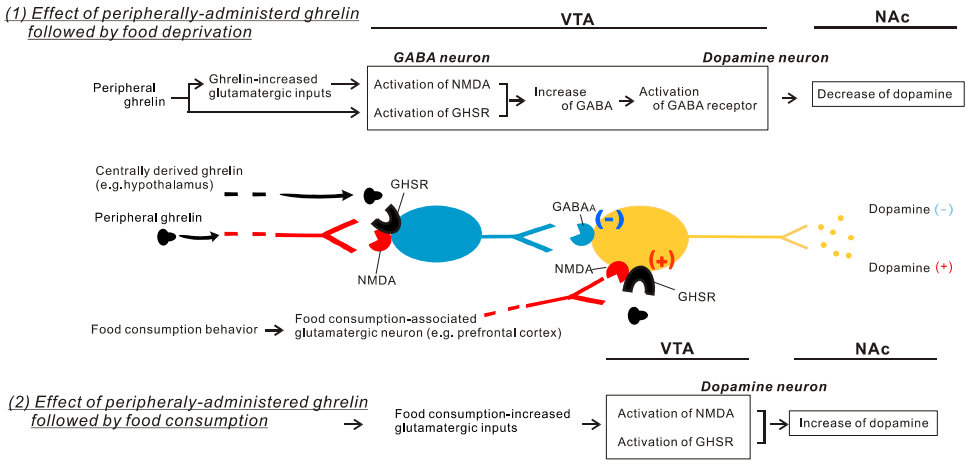

Figure 1. Proposed model of the neuronal interactions that mediate the effect of peripherally -administered ghrelin on the mesolimbic dopamine neurotransmission in different feeding states. Feeding states determine the functional significance of GHSRs localized on either GABA interneurons or dopamine neurons. (1) Peripheral ghrelin followed by food deprivation increases glutamatergic inputs into the VTA via activation of hypothalamic area. Ghrelin possibly derived from brain (e.g. hypothalamus) activates GHSR in the VTA. Activation of GHSR and NMDA receptors localized on GABA interneurons increases the inhibitory tone on dopamine neurons, resulting in the inhibition of the dopamine neuron and release. (2) Peripheral ghrelin followed by food consumption increases glutamatergic inputs into the VTA via activation of different source of glutamatergic neuron (e.g. prefrontal cortex). Activation of GHSR and NMDA receptors localized on dopamine neurons in the VTA results in the stimulation of the dopamine neuron and release. Red line indicates glutamatergic neuron. Orange circle and lines indicate dopamine neurons. Black symbol (●) indicates ghrelin.

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Neurochemical profile of orexin receptor antagonists: awakening the neurochemistry of sleep

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Introduction

The orexins (OX-A and OX-B) are hypothalamic peptides and are known to exert a prominent role in the maintenance of wakefulness. Orexin producing neurons are localized in the dorsal, lateral and posterior hypothalamic area, which project to multiple target fields both within and outside the hypothalamus. The orexins bind and activate two closely related G-protein coupled receptors, orexin-1 (OX_1R) and orexin-2 (OX_2R) re ceptors. OX1R and OX2R are differentially distributed throughout the brain. Within the hypothalamus, a low level of OX1R is observed in the dorsomedial hypothalamus; in contrast the ORX2 receptor is abundantly expressed throughout the hypothalamus (arcuate nucleus, paraventricular nucleus, tuberomammilary nucleus). Locus coeruleus neurons exclusively express OX1R. The raphe nucleus and ventral tegmental area express both OX1R and OX2R. OX2R is also expressed in cerebral cortex, nucleus accumbens, hippocampus and thalamus. The distinct localization of the orexin-1 and orexin-2 receptors suggests interaction with different neurotransmitter systems and thus it is important to understand the impact of different pharmacological manipulations on brain neurochemistry.

Activation of orexin neurons contributes to the promotion or maintenance of wakefulness. It has been demonstrated that the arousal effect of orexin A depends on activation of the histaminergic system [1]. The lack of selective orexin receptor antagonists has hampered the pharmacological investigation of the role of the brain orexin system. Recently, several pharmacological compounds varying selectivity of have become available. JNJ-10397049 [2], a selective OX2R antagonist and ACT-078573 [3], a dual OX1R/OX2R antagonist, have been shown to promote sleep in rodents [3, 4]. The selective OX1R antagonist SB-408124 [5] did not promote sleep in rodent [4]. ACT-078573 also promotes sleep in human during the active phase [3].

Here we compare the neurochemical profile of the selective OX2R antagonist (JNJ-10397049), the dual OXR1/2 antagonist (ACT-078573) and the selective OX1R antagonist (SB-408124) in freely moving rats. Histamine (HA) release was measured in the lateral hypothalamus of freely moving rats during the dark phase. HA was robustly detected using LC/MS/MS. Dopamine (DA) and norepinephrine (NE) levels were measured in the cortex using HPLC/ECD.

Methods

Cannulated naïve male Sprague Dawley rats weighing 300-350g received either vehicle, JNJ-10397049, ACT-078573 or SB-408124 (30 mg/kg) administered s.c. Thirty-minute samples were collected over 12 hours. Animals were dosed 30 minutes prior to the onset of the dark phase. Values were not corrected for *in-vitro* recovery. Dialysate was quantified for HA using LC/MS/MS. A Supelco Discovery HS F5, 2.1 X 100 mm, 3µm column was used with a mobile phase containing a 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The mobile phase was held on the B:A ratio of 100:0 for 1.5 min. The linear gradient was changed over the next 0.5 min to B:A ratio of 5:95 and held for 2.5 min, then returned to the starting conditions. The total run time was 6.5 min and flow rate was 0.6 mL/min (Shimadzu LC-10AD VP with SCL-10A VP system controller). Tandem mass spectrometric (MS/MS) detection was carried out on a PE Sciex API4000 in the positive ion mode (ESI) by multiple reaction monitoring (MH^+/daughter was 112.09 ® 95.1 m/z).

Results and Discussion

Both the selective OX2R antagonist JNJ-10397049 and the dual OX1R/OX2R antagonist ACT-078573 significantly decreased extracellular HA release in the lateral hypothalamus of freely moving rats within 60 minutes post dosing during the dark phase (Fig 1 A, A', B, B'). This suppression was maintained over the twelve- hour study. In contrast, the selective OX1R, SB-408124 had no significant effect on extracellular HA release (Fig. 1C, C'). These neurochemical data suggest that blockade of OX1R is not directly involved in the sleep promoting effects observed with ACT-078573 in agreement with our previous EEG study [4].

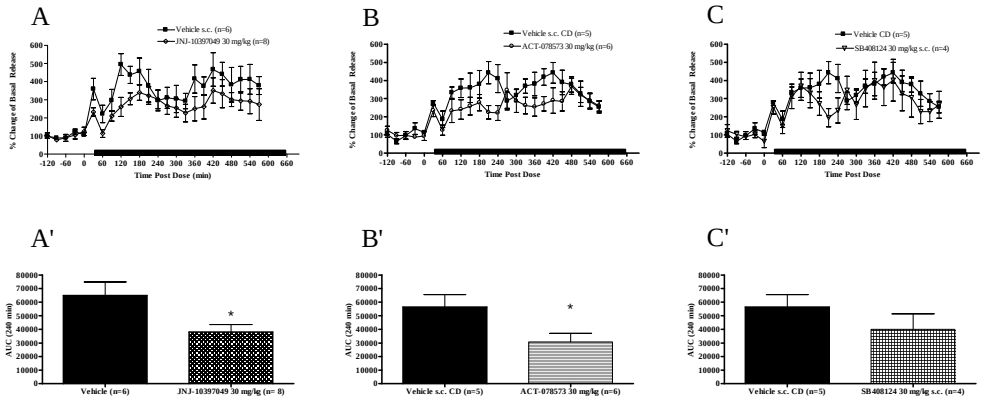

Figure 1. Effect of JNJ-10397049 (A,A'), ACT-078573 (B,B') and SB-408124 (C,C') on extracellular HA release from the lateral hypothalamus of freely moving rats. A, B, C. HA levels are expressed as a percentage of the pre-dose baseline of each animal. Area under the curve are presented in values A', B', C'. Data are presented as mean ± S.E.M. of 4-8 animals.

None of the three compounds modified extracellular NE release in frontal cortex (data not shown). Interestingly, both ACT-078573 and SB-408124 moderate increased extracellular DA release in frontal cortex. In contrast the selective OX2R antagonist JNJ-10397049 did not modify extracellular DA release in frontal cortex (data not shown). These observations indicate that blockade of OX1R might increase DA release in frontal cortex. This may explain the more robust effects observed on latency to non-REM observed with a selective OX2R antagonist compared to an OX1R/OX2R antagonist.

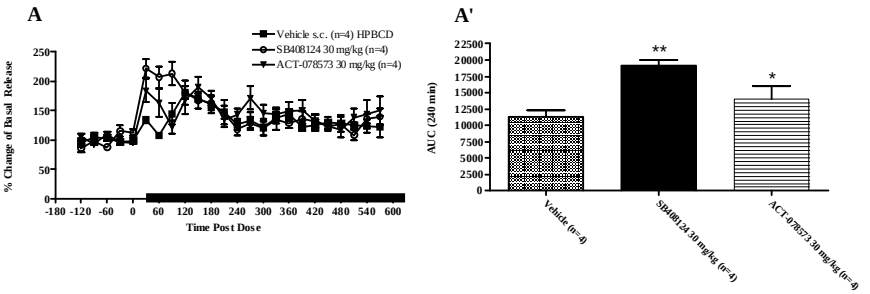

Figure 2. Effect of ACT-078573 and SB-408124 (30 mg//kg) on extracellular DA release from the prefrontal cortex of freely moving rats. DA levels are expressed as a percentage of the pre-dose baseline of each animal (A). Area under the curve values are presented in A'. Data are presented as mean ± S.E.M. of 4 animals.

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