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N-acyldopamines control striatal input terminals via novel ligand-gated cation channels

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ABSTRACT

Endogenous analogues of capsaicin, *N*-acyldopamines, were previously identified from striatal extracts, but the putative presynaptic role of their receptor, the $TRPV_1R$ (formerly: vanilloid or capsaicin receptor) in the caudate–putamen is unclear. We found that the endogenous $TRPV_1R$ agonists, *N*-arachidonoyldopamine (NADA) and oleoyldopamine (OLDA) with EC_{50} values in the nanomolar range, as well as the synthetic $TRPV_1R$ activator 2-aminoethoxydiphenylborane (2APB), and palmytoyldopamine (PALDA, another endogenous *N*-acyldopamine inactive at the $TRPV_1R$), but not capsaicin or other endogenous and synthetic cannabinoids, triggered a rapid Ca^{2+} entry with the concomitant stimulation of glutamate and dopamine release. These effects persisted in the $TRPV_1R$ null-mutant mice, and were insensitive to antagonists of common ionotropic receptors, to several $TRPV_1R$ antagonists and to the absence of K^+. Furthermore, these *N*-acyldopamine receptors in glutamatergic and dopaminergic terminals are different based on their different sensitivity to anandamide, capsazepine and Gd^{3+} at nanomolar concentrations. Altogether, novel ion channels instead of the $TRPV_1R$ mediate the presynaptic action of *N*-acyldopamines in the striatum of adult rodents.

1. Introduction

The transient receptor potential vanilloid type-1 receptor ($TRPV_1R$) is a non-selective Ca^{2+} and Na^+ channel activated by heat, protons, inflammatory agents, endovanilloids and the pungent substance, capsaicin of chilli pepper, and is involved in peripheral and central pain transmission and procession (Caterina et al., 1997; Avelino and Cruz, 2006; Nagy et al., 2008). The so-called endovanilloids are diverse lipidomics (mostly arachidonate-derivatives) (Starowicz et al., 2007), and among them, perhaps anandamide (arachidonoylethanolamine, AEA, Fig. 1C) is the most studied as it also activates the inhibitory metabotropic type-1 cannabinoid receptor (CB_1R) (Devane et al., 1992; Lovinger, 2008). *N*-arachidonoyldopamine (NADA) is another endogenous ligand capable of activating both the CB_1R and the $TRPV_1R$, and belongs to the recently described family of *N*-acyldopamines (Starowicz et al., 2007). Several similar fatty acid dopamide conjugates have been identified in striatal extracts, among which NADA and *N*-oleoyldopamine (OLDA) (Fig. 1C) are as efficacious and potent as capsaicin at the $TRPV_1R$ (Huang et al., 2002; Chu et al., 2003). However, the observation that palmytoyldopamine (PALDA; Fig. 1C) lacks agonist activity at the $TRPV_1R$ (Chu et al., 2003) prompts the hypothesis that *N*-acyldopamines may have additional targets.

In the brain, $TRPV_1Rs$ display higher density in the cortex, the hippocampus, the preoptic area, the medial basal hypothalamus, the locus coeruleus and the basal ganglia (Mezey et al., 2000; Sanchez et al., 2001; Szabó et al., 2002; Roberts et al., 2004; Tóth et al., 2005; Cristino et al., 2006). Marinelli et al. (2003, 2005, 2007) found that $TRPV_1R$ activation can directly stimulate dopamine release or glutamatergic transmission in the substantia nigra pars compacta, the ventral tegmental area and the nucleus accumbens of the rat. In the striatum, presynaptic $TRPV_1Rs$ desensitize rapidly, but the activation of protein kinase C (PKC) unmasks $TRPV_1R$-mediated presynaptic facilitatory actions (Musella et al., 2009). Thus, in the absence of PKC activation, predominantly post-synaptic $TRPV_1R$ activation can be detected which down-regulates the post-synaptic release of 2-arachidonoylglycerol (2-AG) (Maccarrone et al., 2008). Importantly, 2-AG (Fig. 1C) is another

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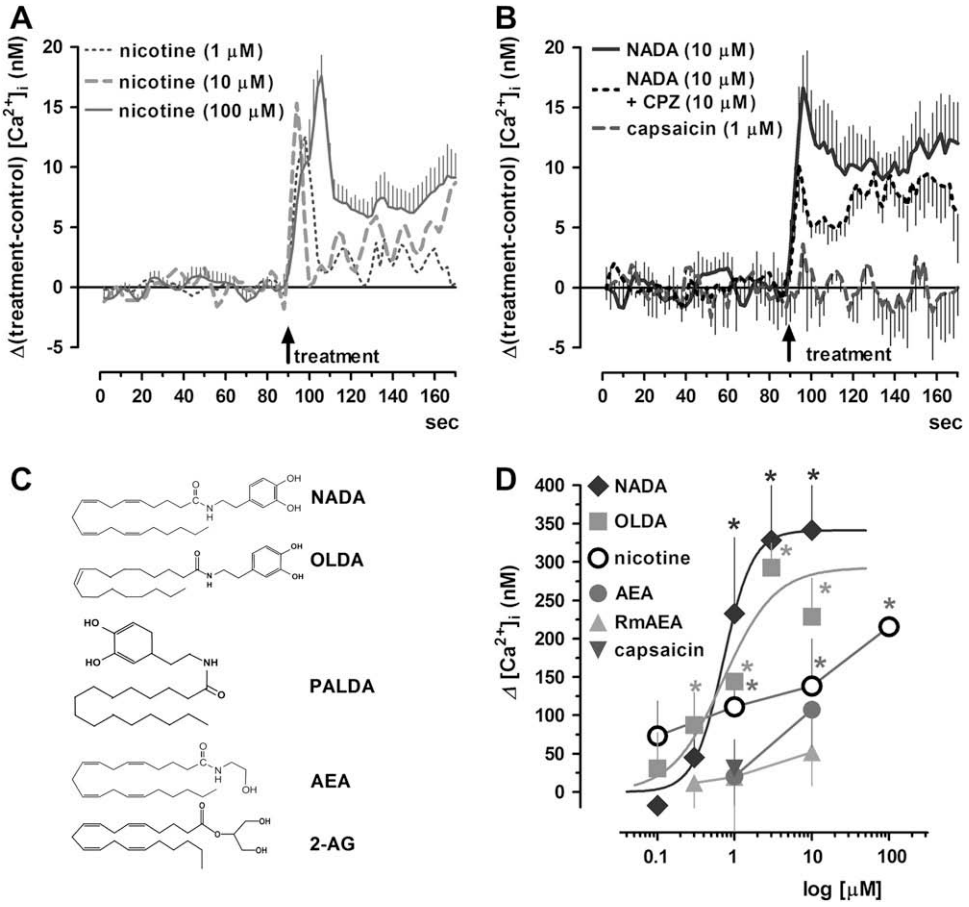

Fig. 1. Nicotine and *N*-acyldopamines induce Ca^{2+} transients in striatal nerve terminals. Nerve terminals were loaded with Fura2/AM, and left for 4 min in the cuvette for stabilization under stirring. The first 90 s represents the pre-treatment period, and subsequently, 2 μl of vehicle or drugs was administered. Emission values were collected at 2-s intervals and expressed as nM. The average of the two everyday control values was subtracted from the treatments and displayed as net drug effect in panels A and B. A) The classical striatal excitant, nicotine, triggers rapid $[Ca^{2+}]_i$ rise in a concentration-dependent fashion. For the sake of simplicity, only the average of the nicotine (100 μM) experiments is presented with error bars (panel A). B) The endogenous $TRPV_1R$ agonist, NADA (continuous line) but not the botanical $TRPV_1R$ activator, capsaicin (dashed line) triggers rapid $[Ca^{2+}]_i$ which is only partially prevented by the $TRPV_1R$ antagonist, capsazepine (CPZ, 10 μM), dotted line. C) Chemical structure of *N*-acyldopamines and endocannabinoids used throughout the study. D) Concentration–response curves for ligands tested in the fluorimetric assay. Note that 2-AG and WIN55212-2 absorb more light at 340 than at 380 nm falsely diminishing Ca^{2+} level readings, therefore, these values are not displayed in the graph (for more information, see Section 2, Table 1, and the 2nd paragraph of Section 3). All points represent mean ± S.E.M. of $n \geq 6$ different observations in duplicate. $^*p < 0.05$.

major CB_1R agonist functioning as a retrograde messenger in the synapse (Stella et al., 1997; Lovinger, 2008); hence post-synaptic $TRPV_1R$ activation may set free glutamatergic terminals from CB_1R-mediated inhibitory control.

Based on these observations, one would expect that $TRPV_1R$ activation causes hyperkinesia either by decreasing 2-AG release locally in the striatum or by increasing glutamate and/or dopamine release via the activation of the striatal afferents. Surprisingly, the opposite is the case: $TRPV_1R$ agonists, either systemically or locally administered, induce hypokinesia and decrease nigrostriatal activity and striatal DOPAC content, inasmuch as $TRPV_1R$ antagonists induce hyperactivity (Di Marzo et al., 2001; de Lago et al., 2004; Tzavara et al., 2006). Thus, these controversial data invite further studies since the role of the endovanilloid system in neuromodulation and in neurological and psychiatric disorders has been emerging.

In this present study, we aimed at analyzing the presynaptic role of capsaicin, anandamide, NADA and OLDA in a relevant brain area, namely, the striatum. We used nicotine as a positive control, and PALDA as a negative one. Our goal was to monitor the dynamics of intrasynaptosomal Ca^{2+} level changes upon administration of vanilloid ligands, together with their potency and efficacy to induce glutamate and dopamine release.

2. Methods

2.1. Preparation of synaptosomes

All studies were conducted in accordance with the principles and procedures outlined in the EU guidelines and were approved by the local Animal Care Committee of the Institute. All efforts were made to reduce the number of animals used and to minimize their suffering. Therefore all rats (male Wistar rats, 140–160 g, 6–8-week old, vendor: Charles-River, Barcelona, Spain) and wild-type mice (C57bl/6, 10–12-week old, vendor: Charles-River, Barcelona, Spain) and genotyped $TRPV_1R$ null-mutant mice (C57bl/6, 10–12-week old, vendor: The Jackson Laboratory) were anesthetized with halothane before being decapitated. Striata from two rats (fluorimetry) or one rat or one mouse ($^{45}Ca^{2+}$ uptake assay and [^{14}C]glutamate and [^{3}H]DA release assay) were quickly removed into ice-cold sucrose solution (0.32 M, containing 5 mM HEPES, pH 7.4) and were homogenized instantly with a Teflon homogenizer, and centrifuged at 5000 *g* for 4 min. The supernatant was centrifuged at 13,000 *g* for 10 min. The mitochodrium-free fraction of the pellet was collected and washed at 13,000 *g* for 2 min in 45% Percoll solution at 4 °C, then decanted and stored sealed on ice.

2.2. Fluorimetric assay

Experiments were performed as described by us earlier (Köfalvi et al., 2007): synaptosomal pellets (~1 mg protein) were preincubated with Fura2/AM (5 μM) for 15 min at 25 °C in the incubation solution of the following composition: NaCl (132 mM), KCl (1 mM), $MgCl_2$ (1 mM), $CaCl_2$ (0.1 mM), H_3PO_4 (1.2 mM), glucose (10 mM), HEPES (10 mM), pH 7.4. Then the pellet was centrifuged at 13,000 *g*,

washed and resuspended in 2 ml assay solution [NaCl (132 mM), KCl (3.1 mM), $MgCl_2$ (1.2 mM), $CaCl_2$ (2.5 mM), H_3PO_4 (0.4 mM), glucose (10 mM), HEPES (10 mM), pH 7.4]. After the 4 min stabilization period at 37 °C, data were collected with 2-s intervals by a computer-assisted spectrofluorometer (Fluoromax, Spex Industries, Edison, NJ) at 510 nm emission and double excitation at 340 and 380 nm, with 1.175 nm slits. The first 90 s represented the pre-treatment period, and then 2 μl of the stock drug solutions was applied. The calibration was done 70 s later, using 5 μM ionomycin (R_{max}), and 30 s later, 10 mM EGTA/30 mM TRIS, pH 9.6 (R_{min}). The fluorescence intensities were converted into $[Ca^{2+}]_i$ values by using the calibration equation for double excitation wavelength measurements and taking the dissociation constant of the Fura2/Ca^{2+} complex as 224 nM (Grynkiewicz et al., 1985).

2.3. Calculation

In each experiment, vehicle controls and treatments were performed in duplicate. The average of the daily vehicle controls was subtracted from the average of the treatments. Then, a forecast line was fitted to the first 45 data points (representing the 90 s pre-treatment period) with linear regression. Drug effects were calculated as the change in $[Ca^{2+}]_i$ (nM)/min, compared to the forecast line with the area under the curve method.

2.4. Effect of drugs on absorbance and emission

Table 1 summarizes the absorbance and the emission values of tested ligands at the highest used concentrations. Capsaicin (1 μM) and 2-arachidonoylglycerol (2-AG, up to 3 μM) do not absorb at 340 and 380 nm; anandamide (AEA, 10 μM), *R*-methanandamide (RmAEA, 10 μM), and *N*-arachidonoyldopamine (NADA, 10 μM) significantly but equally absorb at the two wavelengths resulting in ratios not significantly different from ethanol control. However, the CB_1R agonists, WIN55212-2 (1 μM: by 18.2 ± 0.24%; 10 μM: by 45.1 ± 0.12%; $n = 6$, $p < 0.001$) and 2-AG (10 μM: by 4.1 ± 0.29%; $n = 6$, $p < 0.001$) already significantly absorbed more at 340 nM diminishing the $Intensity_{340}/Intensity_{380}$. As a consequence, when applied in the cuvette, WIN55212-2 and 2-AG (10 μM) induced an artificial decrease of in the $Fura2_{bound}/Fura2_{free}$ ratio which had to be corrected during the calculation of $[Ca^{2+}]_i$, based on the equation of Grynkiewicz et al. (1985). The emission of WIN55212-2 and 2-AG was determined by the use of a Spex spectrofluorometer. Cuvettes were filled with 2 ml of milliQ water to determine the emission of 510 nm light at 340 and 380 nm excitation, and subsequently vehicle (ethanol) or the tested compound (with the same amount of vehicle) was applied, and results from six independent observations were averaged. As a consequence that 2-AG significantly absorbs more light at 340 than at 380 nm it decreases the Em_{340}/Em_{380} ratio significantly and concomitantly, falsely diminishes $[Ca^{2+}]_i$ readings.

2.5. $^{45}Ca^{2+}$ uptake assay

Experiments were carried out in the same manner (timing, conditions, and assay solutions) as in the fluorimetric assay. Synaptosomal aliquots of 50 μl (~1–2 mg) were diluted to 600 μl in the assay solution and kept at 37 °C for 5 min. Then 400 μl of pre-warmed assay solution containing $^{45}Ca^{2+}$ (isotope purchased from PerkinElmer) and the vehicle or drug tested was added to the synaptosomes. The final concentration of the isotope was 0.5 μCi/ml. Sixty second later, 1 ml of ice-cold Ca^{2+}-free assay solution containing 10 mM EGTA/30 mM TRIS (pH 9.6) was added to stop reactions and the samples were vacuum-filtered immediately in GF/B filters. The $[^{45}Ca]$ content of each sample was counted by a Tricarb β-counter (PerkinElmer), and expressed as $\Delta[Ca^{2+}]$ (nM/mg protein).

Table 1
Optical density values at 340 and 380 nm and emission values at 510 nm wavelengths of cannabinoid and vanilloid ligands used in fluorimetry.

Treatment	OD_{340}	OD_{380}	Ratio
CTRL (vehicle)	52.0 ± 0.8	41.7 ± 0.2	100%
AEA (10 μM)	76.7 ± 1.6*	61.7 ± 1.4*	100.3% n.s.
RmAEA (10 μM)	71.7 ± 2.2*	57.3 ± 1.8*	99.8% n.s.
2-AG (3 μM)	51.7 ± 0.6	41.0 ± 0.7	100.3% n.s.
2-AG (10 μM)	65.7 ± 0.6*	49.3 ± 0.5*	94.9%*
WIN (0.3 μM)	50.0 ± 0.4	39.0 ± 0.4	98.6%
WIN (1 μM)	69.0 ± 0.4*	44.7 ± 0.2*	81.8%*
WIN (10 μM)	128.8 ± 0.4*	57.7 ± 0.2*	56.9%*
2APB (100 μM)	49.5 ± 0.8	40.3 ± 0.9	101.0% n.s.
NADA (10 μM)	137.0 ± 2.3*	112.0 ± 0.4*	102.0% n.s.
OLDA (10 μM)	55.0 ± 1.6	43.7 ± 1.0	98.6% n.s.
PALDA (10 μM)	52.0 ± 0.8*	42.3 ± 0.6*	99.0% n.s.
Capsaicin (1 μM)	49.3 ± 0.8	39.7 ± 1.0	100.2% n.s.
Treatment	Em_{340} % of CTRL	Em_{380} % of CTRL	Ratio
CTRL (vehicle)	100% (3942 ± 91 cpm)	100% (3117 ± 67 cpm)	100%
2-AG (10 μM)	167.1 ± 1.0%*	193.1 ± 2.4%*	86.6%*

*$P < 0.05$

2.6. Dual-label [^{3}H]DA/[^{14}C]glutamate release assay from striatal nerve terminals

Experiments were carried out as before (Köfalvi et al., 2007). Striatal synaptosomes were diluted to 3 ml with Krebs' solution (in mM: NaCl 113, KCl 3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 10, oxygenated with 95% O_2, and 5% CO_2, pH 7.4, 37 °C), and equilibrated for 5 min. After this, 20 μCi of [^{14}C]glutamate (PerkinElmer) and 10 μCi of [^{3}H]dopamine (GE Healthcare) were added to the synaptosomes for 10 min. All solutions contained the glutamate decarboxylase inhibitor aminooxyacetic acid (100 μM) and the MAO-B inhibitor pargyline (10 μM). A 16-microvolume chamber perfusion setup was filled with the preloaded synaptosomes which were trapped by layers of Whatman GF/C filters and superfused continuously at a rate of 0.7 ml/min until the end of the experiment with oxygenated Krebs' solution at 37 °C. Upon termination of the 15-min washout, 2-min samples were collected for liquid scintillation assay, and the filters were also harvested to obtain the total radioactivity content. In each experiment, treatments were applied in quadruplicate (*i.e.* 4 chambers served as control averaged as $n = 1$ and three other treatments were applied in the rest of the 3-times 4 chambers). All drugs were perfused from the 6th min of sample collection for 3 min. High K^+ was applied as isomolar substitution of Na^+ by K^+ in the buffer. The proportion of dopamine and glutamate in the released radioactivity was confirmed to be >95%, as previously reported (Köfalvi et al., 2007). The [^{14}C] and [^{3}H] contents of each samples were counted by dual-label protocol by a Tricarb β-counter (PerkinElmer), and DPM values were expressed as fractional release (FR%), *i.e.* the percent of actual content in the effluent in the function of the total synaptosomal content (for details see Köfalvi et al., 2007).

2.7. Data treatment

All data represent mean ± SEM of $n \geq 5$ observations. After subtracting the respective control value from the effect of treatment, statistical significance was calculated by column statistics (difference from the hypothetical value of zero if the question was whether a treatment has had any effect or not), Student's *t*-test or ANOVA followed by Bonferroni's test. A $p < 0.05$ was accepted as significant difference.

2.8. Drugs

HEPES, WIN55212-2, sucrose, aminooxyacetic acid, nicotine, sulpiride, and ionomycin were obtained from Sigma, Saint Louis, Missouri, USA. Arachidonoylethanolamide (anandamide, AEA), 2-arachidonoylglycerol (2-AG), SB366791, 2-aminoethoxydiphenylborane (2APB), Ruthenium Red, iodoresiniferatoxin (IRTX), AM251, (+)-tubocurarine chloride, and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid tetrasodium salt (PPADS) were obtained from Tocris Bioscience, UK. *N*-arachidonoyldopamine (NADA), *N*-oleoyldopamine (OLDA), palmytoyldopamine (PALDA), nifedipine, D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5), CNQX, and (−)-bicuculline methobromide were purchased from Ascent Scientific, UK. Fura2/AM was purchased from Alfagene (Lisbon, Portugal). Non-water soluble substances were dissolved or reconstituted in ethanol at different concentrations and aliquoted and stored at −20 °C. Stocks of cannabinoid and vanilloid agonists to be tested were always diluted by 1000 times to have always the same concentration of vehicle in the buffer.

3. Results

3.1. Validation of the fluorimetric assay

In rat striatal nerve terminals, the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) was 101.9 ± 8.5 nM ($n = 76$) in the beginning of the recording in accordance with previous studies both in whole cells and in nerve terminals (Lauckner et al., 2005; Köfalvi et al., 2007). To test the functionality of presynaptic ligand-gated ion channels in our experimental model we used nicotine, the classical agonist of choice in the striatum (Zhou et al., 2002). Nicotine (1–100 μM) induced a rapidly developing and desensitizing Ca^{2+} transient in a concentration-dependent fashion followed by a sustained but lower elevation of $[Ca^{2+}]_i$ at higher nicotine concentrations (Fig. 1A,D).

3.2. N-*acyldopamines, but not classical TRPV$_1$R and/or CB$_1$R agonists,* per se *induce* $[Ca^{2+}]_i$ *rise*

NADA (1–10 μM) and OLDA (0.3–10 μM) triggered an immediate, non-desensitizing and concentration-dependent $[Ca^{2+}]_i$ rise. Both substances reached the maximum effect at the concentration of

3 μM, and surprisingly, both displayed the same EC_{50} value (713 nM; calculated from the best-fit variable slope sigmoid curve) (Fig. 1B,D). The $TRPV_1R$ antagonist, capsazepine (10 μM, added 4 min before recording) significantly diminished the NADA (10 μM)-evoked $[Ca^{2+}]_i$ rise by 29.2 ± 5.2%, $n = 6$, $p < 0.05$ by ANOVA of repeated measures test (Fig. 1B). Anandamide (AEA, 1–10 μM) and its non-metabolizing analogue, *R*-methanandamide (RmAEA 0.1–10 μM) as well as capsaicin (1 μM) did not significantly affect $[Ca^{2+}]_i$ up to the maximally tested concentration of 10 μM, though $\Delta[Ca^{2+}]_i$ in the presence of AEA (10 μM) was at the verge of significance ($p = 0.0504$; $n = 11$ animals). As mentioned in Section 2, the CB_1R agonists, WIN55212-2 (from 1 μM) and 2-AG (at 10 μM) significantly reduce the Em_{340}/Em_{380} ratio. Therefore, we needed to re-calculate their effect on the $[Ca^{2+}]_i$ with modifications of the Grynkiewicz equation. According to this calculation, 2-AG and WIN55212-2 (0.03–10 μM) also failed to affect the $[Ca^{2+}]_i$ (data not shown).

3.3. N-acyldopamines trigger $^{45}Ca^{2+}$ uptake in striatal nerve terminals

This assay was performed A) to test whether the $[Ca^{2+}]_i$ rise represents Ca^{2+} entry from the extracellular buffer or originates from intracellular Ca^{2+} stores; B) to validate the $\Delta[Ca^{2+}]_i$ values with direct measurement; and C) to confirm our calculations on the lack of effect of 2-AG and WIN55212-2. Both NADA (10 μM) and OLDA (10 μM) induced significant Ca^{2+} uptake with values similar to those found with the fluorimetric assay (Fig. 2). Interestingly, PALDA (10 μM), which is inactive at the $TRPV_1R$, also induced a Ca^{2+} uptake of similar size. 2APB (100 μM), which is a sensitizer (≤100 μM) and an activator (≥100 μM) of the homomeric $TRPV_1R$, $TRPV_2R$, and $TRPV_3R$ (Hu et al., 2004), also induced Ca^{2+} uptake twice as large as NADA. Neither the hybrid $TRPV_1R/CB_1R$ agonists, anandamide (10 μM) and *R*-methanandamide (10 μM) nor the CB_1R agonists, 2-AG (10 μM) and the synthetic WIN55212-2 (1 μM) triggered statistically significant Ca^{2+} uptake.

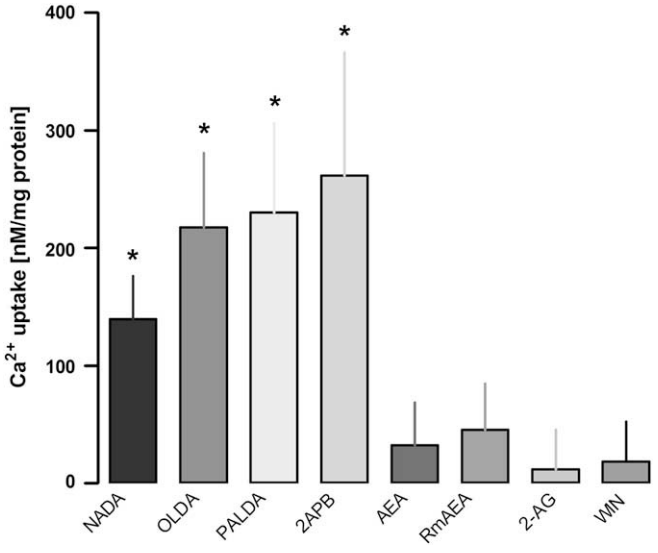

Fig. 2. *N*-acyldopamines (each at 10 μM) and 2APB (100 μM) induce Ca^{2+} uptake into striatal nerve terminals. The rest of the ligands was tested also at 10 μM except for WIN55212-2 (WIN, 1 μM). All solutions and protocols were identical to those of the fluorimetric assay. Upon termination of the experiment, reactions were stopped with addition of 10 mM EGTA/30 mM TRIS (pH 9.6), then synaptosomes were immediately vacuum-filtered and filters were counted for $^{45}Ca^{2+}$. The obtained values were then converted into total (that is, radioactive + cold) Ca^{2+} uptake values represented by the figure. Values are mean + S.E.M. of ≥6 rats; $^*p < 0.05$.

3.4. N-acyldopamines trigger the release of dopamine and glutamate

To validate this neurochemical procedure, K^+ and nicotine were employed to stimulate transmitter release. Both K^+ (10 and 15 mM) and nicotine (100 μM) induced a rapidly developing dopamine and glutamate release which returned to baseline upon washout (Fig. 3A,B). Total amounts of released dopamine and glutamate upon drug superfusion are displayed in Fig. 4. K^+ triggered the release of glutamate and dopamine to a similar extent, while nicotine mostly affected the release dopamine (yet, its effect on the release of glutamate was also statistically significant) (Fig. 3A,B and Fig. 4A).

In agreement with the fluorimetric and $^{45}Ca^{2+}$ uptake data, NADA, OLDA, PALDA (from as low as 1 μM) and 2APB (100 μM, the only concentration tested) triggered the release of [^{3}H]dopamine and [^{14}C]glutamate from striatal nerve terminals with the order of efficacy of OLDA ≈ NADA > 2APB ≈ PALDA. At the concentration of 100 nM none of the three *N*-acyldopamines induced significant transmitter release (data not shown). Capsaicin (1 and 10 μM) had no effect on the release of either transmitter (Fig. 5B). Next, we tested if NADA's effect involves TASK-3 K^+ channel blockade with the concomitant depolarization, Ca^{2+} entry and transmitter release – as hypothesized before (Köfalvi et al., 2007). Yet, in K^+-free medium, the effect of NADA was unchanged (Fig. 4B). Somewhat surprisingly, the nicotine-evoked release of dopamine (Fig. 4A) decreased by 53% ($n = 5$, $p < 0.05$) but not that of glutamate ($n = 5$, $p > 0.05$) in the absence of K^+.

3.5. Inhibitors of TRPV channels, the CB_1 receptor, and common ionotropic receptors fail to modify the NADA-induced release of dopamine and glutamate

Although the ion channels involved in the endovanilloids' action display a mixed $TRPV_1R$ and non-$TRPV_1R$ pharmacology neither the selective and potent $TRPV_1R$ antagonists, iodoresiniferatoxin (IRTX, 1 μM) and SB366791 (1 μM) nor the non-selective $TRPV_1$, $TRPV_2$, $TRPV_3$ and $TRPV_4$ channel blocker Ruthenium Red (10 μM) antagonized the effect of NADA (Fig. 4B). In contrast to hippocampal nerve terminals (Köfalvi et al., 2007) Ruthenium Red did not induce transmitter release alone indicating the lack of TASK-3 K^+ channels in striatal dopaminergic and glutamatergic terminals (data not shown). The putative role of another class of voltage-gated cation channels, the L-type Ca^{2+} channels was investigated with the Ca^{2+} channel blocker nifedipine (1 μM) which did not alter the amplitude of NADA's effect (Fig. 4B). As NADA being a dual $CB_1R/TRPV_1R$ agonist, we investigated tested NADA (10 μM) under CB_1R blockade by the CB_1R-selective antagonist, AM251 (1 μM). AM251 did not significantly change the amplitude of NADA's effect on the release of dopamine and caused a non-significant ($p = 0.106$) facilitation on the release of glutamate ($n = 6$, Fig. 4B). Ionotropic glutamate receptor antagonists, CNQX (50 μM) and AP-5 (10 μM); the nicotinic and 5-HT_3 receptor antagonist, tubocurarine (10 μM); the $GABA_A$ receptor antagonist, bicuculline (10 μM); and the P2X receptor antagonist, PPADS (10 μM) also failed to modify the action of NADA (Fig. 4C).

3.6. Anandamide, capsazepine and Gd^{3+} reveal different NADA receptors

Interestingly, anandamide (10 μM) triggered a statistically significant (though very little) release of dopamine (Fig. 3C, Fig. 5B) but not that of glutamate (Fig. 3D). Furthermore, capsazepine, which is a low-potency $TRPV_1R$ antagonist thought to be selective over the other TRP channels, attenuated the effect of NADA on the release of dopamine by 59% ($n = 6$, $p < 0.05$ by ANOVA of repeated

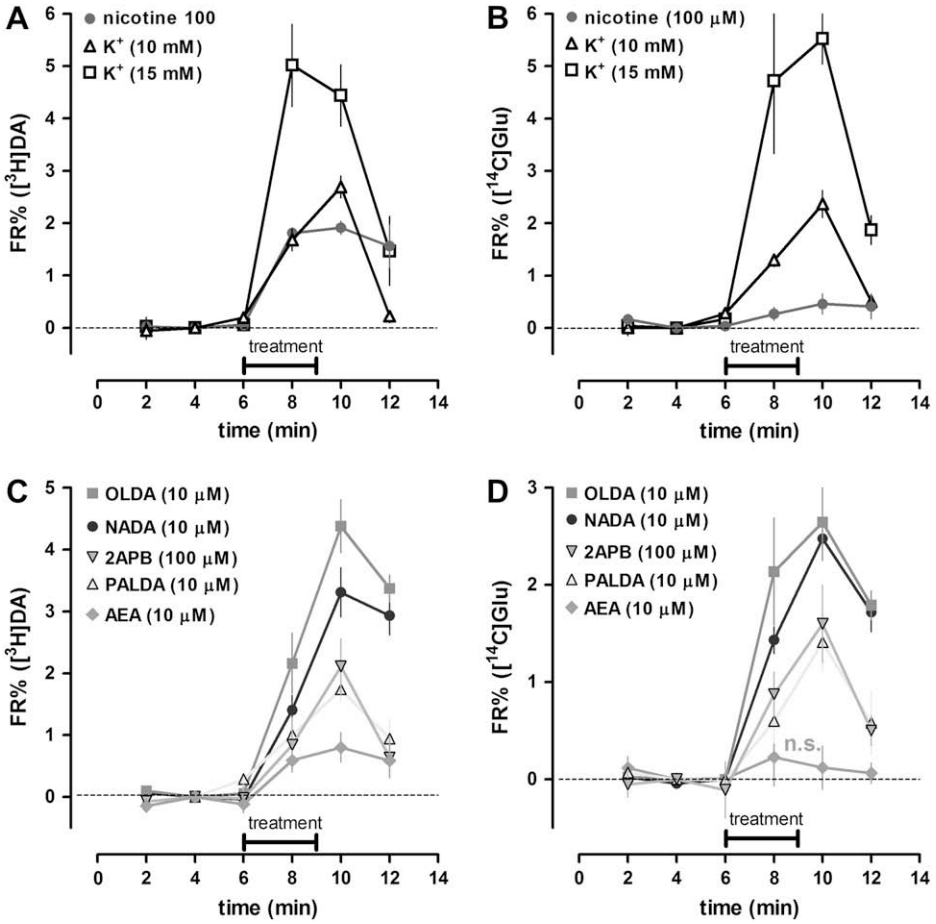

Fig. 3. Release diagrams for the effect of K^+, nicotine, and TRPV ligands on the release of [^{3}H]dopamine and [^{14}C]glutamate from rat striatal nerve terminals. Drugs or vehicle was administered as indicated by the horizontal bar. An FR% value should be read as the amount of released transmitter as the percentage of the total nerve terminal content. For instance, a value of 10% means that a treatment deliberated 10% of the nerve terminal content. All treatments resulted in significant transmitter release except for AEA on the release of glutamate (denoted as n.s.; also consult with Fig. 4B,C and Fig. 5B). Values are mean + S.E.M. of ≥6 rats.

measures test) but did not modify the effect of NADA on the release of glutamate (Fig. 4B). Recent reports showed that TRPV channels can be inhibited by the trivalent cation Gd^{3+} at mid-micromolar concentrations (as for the $TRPV_1R$, above 100 μM) (Tousova et al., 2005; Leffler et al., 2007). In our study, Gd^{3+} fully abolished the effect of NADA, OLDA, PALDA and 2APB at a concentration as low as 3 μM (Fig. 5A,B). The ultra high potency of Gd^{3+} against NADA's effect on the release on dopamine (IC_{50}, 745 nM) was even surpassed on the release of glutamate (IC_{50}, 153 nM) (Fig. 5A). Gd^{3+} (3 μM) failed to affect the K^+ (15 mM)-evoked release of both transmitters (data not shown).

3.7. The effect of NADA, OLDA, PALDA and 2APB persists in the $TRPV_1R$ null-mutant mice

In the following step, we verified that the effect of the three fatty acid dopamides (all at 10 μM) and of 2APB (100 μM) is present in another species, the wild-type C57bl/6 mice. Additionally, we found that all four ligands triggered the release of glutamate and dopamine in the $TRPV_1R$ null-mutant ("knockout") mice, and the size of effects was not statistically different ($p > 0.05$, $n = 5$) from that in the wild-type (Fig. 6).

4. Discussion

N-acyldopamines, NADA and OLDA, have been so far considered as selective endogenous agonists for the $TRPV_1R$ (formerly: VR1 vanilloid or capsaicin receptor) among ligand-gated ion channels (Huang et al., 2002; Chu et al., 2003). We report now that *N*-acyldopamines, NADA, OLDA and PALDA, posses new presynaptic targets in the striatum which are linked to rapid Ca^{2+} entry and the concomitant release of dopamine and glutamate. The amplitude of effects exceeds that of nicotine and is similar to that of K^+ (15 mM) depolarization, arguing for the significance of this novel presynaptic neuromodulation mechanism in the striatum.

4.1. The suitability of our experimental models

Importantly, we have chosen to measure dopamine and glutamate release as they represent the major input terminals of the complex of the caudate–putamen nuclei. Synaptosomal preparations are the golden standard to test presynaptic neuromodulation devoid of tonic effects and polysynaptic/glial influences (Raiteri and Raiteri, 2000). To test presynaptic ligand-gated ion channel functionality in our experimental models, we utilized nicotine, the classical agonist of choice. Nicotine typically increases striatal dopamine levels and consequently, stimulates locomotor activity in rodents (Zhou et al., 2002). The two classes of nACh receptors mediating nicotine's effect in striatal dopaminergic terminals are formed mostly of α_4, α_5, α_6, β_2, and β_3 subunits (Salminen et al., 2004). In our study, nicotine induced rapid Ca^{2+} transients in a concentration-dependent fashion followed by a sustained but lower elevation of $[Ca^{2+}]_i$ at higher nicotine concentrations. This latter phase may be mediated by a slowly inactivating second

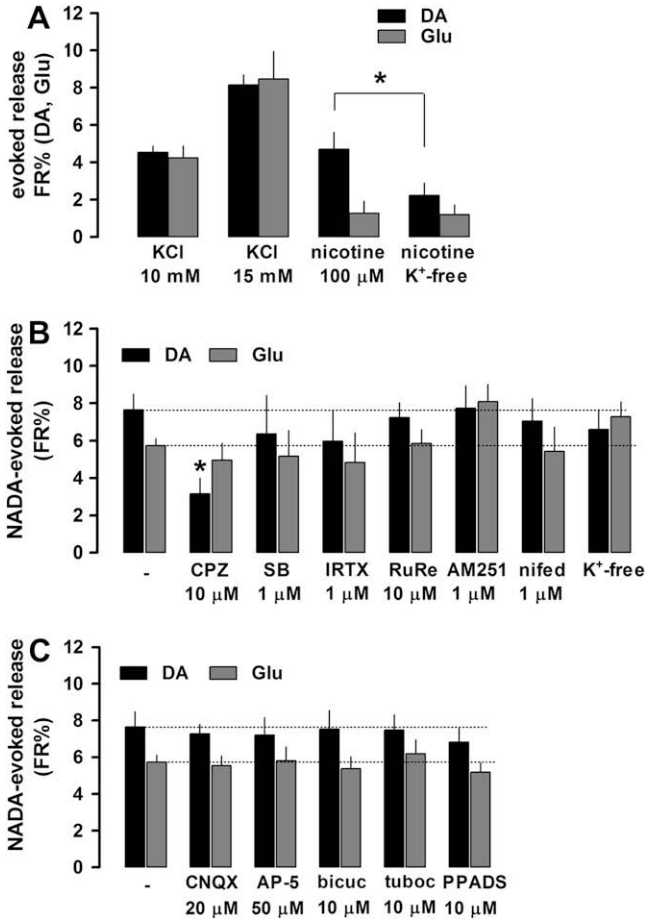

Fig. 4. Sensitivity of the effect of NADA to antagonists of TRPV, CB_1, and common ionotropic receptors, to lack of K^+, and to Ca^{2+} channel blockade. A,B) The NADA (10 μM)-evoked release of dopamine (black bars) and glutamate (grey bars) is greater than that of nicotine (100 μM) and similar to that of K^+ (15 mM). In K^+-free medium, nicotine displayed a reduced efficacy to trigger the release of the two transmitters. Among the TRPV channel antagonists, only capsazepine (CPZ) antagonized the effect of NADA and only on the release of dopamine, while iodoresiniferatoxin (IRTX) and Ruthenium Red (RuRe) failed to do so. AM251 (1 μM), the selective antagonist of the inhibitory metabotropic CB_1R, did not facilitate significantly the effect of NADA on the release of glutamate and also left unaffected the release of dopamine triggered by NADA. The L-type Ca^{2+} channel blocker nifedipine (nifed, 1 μM) did not modify the effect of NADA on either transmitter. The effect of NADA is also unchanged in the absence of K^+. C) The AMPA/kainate receptor antagonist, CNQX, the NMDA receptor antagonist, AP-5, the $GABA_A$ receptor antagonist, bicuculline (bicuc), the nACh receptor and 5-HT_3 receptor antagonist, tubocurarine, and the P2X ATP receptor antagonist, PPADS all failed to counteract the effect of NADA. Values are mean + S.E.M. of = 6 rats = 6; $^*p < 0.05$.

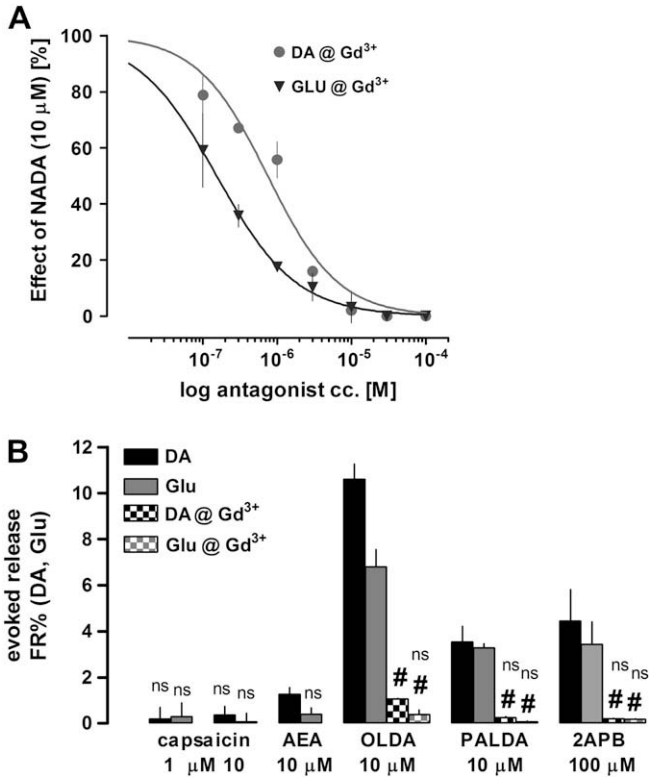

Fig. 5. Sensitivity to anandamide and gadolinium reveals differences between the receptors on dopaminergic and glutamatergic terminals. A) Gd^{3+} very potently inhibits the effect of NADA in a concentration-dependent fashion, with 5-times greater potency on the release of glutamate. B) While capsaicin (1 and 10 μM) failed to trigger transmitter release, anandamide stimulated the efflux of dopamine from striatal nerve terminals. Gd^{3+} (3 μM) abolished the effect of OLDA, PALDA and 2APB, respectively, suggesting that the receptors/ion channels mediating their effects are identical to those of NADA. Symbols: "ns" means that the observed release values were not significantly different from the vehicle control. A lack of symbol above the full bars (black bars, dopamine; grey bars, glutamate) means that the effect of the substance is significantly different from the vehicle. # ($p < 0.01$) denotes that Gd^{3+} statistically significantly inhibited the evoked release. Values are mean + S.E.M. of ≥5 rats.

nAChR class. Or else, the 53% decrease in nicotine's effect on the release of dopamine in K^+-free solution suggests that nAChRs inhibited a slowly inactivating K^+ conductance, resulting in membrane depolarization (Hamon et al., 1997).

Altogether, our neurochemical tools are able to directly demonstrate Ca^{2+} transients and the consequent release of neurotransmitters upon activation of presynaptic ligand-gated ion channels.

4.2. N-*acyldopamines activate striatal input terminals*

NADA and OLDA, the two endogenous agonists of the $TRPV_1R$, being equipotent and equally efficacious to capsaicin, and PALDA, which does not activate the $TRPV_1R$ (Huang et al., 2002; Chu et al., 2003) *per se* triggered Ca^{2+} entry and the release of dopamine and glutamate. Apart from these *N*-acyldopamines, a non-selective enhancer/activator of the $TRPV_1$, $TRPV_2$ and $TRPV_3$ receptors, 2APB (Hu et al., 2004), also triggered Ca^{2+} entry and consequently, the release of both dopamine and glutamate. Somewhat

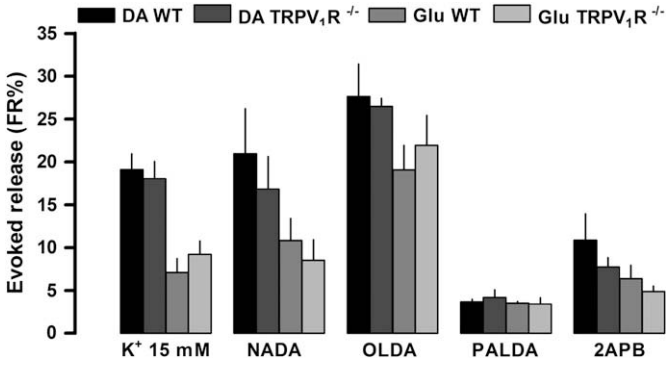

Fig. 6. High K^+, *N*-acyldopamines and 2APB are equally efficacious to trigger the release of dopamine (black bars) and glutamate (grey bars) in the C57bl/6 mice (full bars, $n = 5$) and in the $TRPV_1R$ knockout mice (hatched bars, $n = 5$). Note that while the effect of K^+ (15 mM) is doubled in the mouse compared to the Wistar rat, effect amplitudes for NADA, OLDA and 2APB are 2–2.5-times greater than in the rat, whereas the effect of PALDA is the same (~4% release of the total releasable pool), indicating slight species differences.

contradictorily, 2APB and PALDA triggered twice as large Ca^{2+} uptake but only half as much transmitter release as NADA and OLDA did. Yet, this discrepancy can be easily resolved knowing that 2APB evokes large GABA release (shown so far only in the hippocampus; Köfalvi et al., 2006). As the striatum is the largest GABAergic nucleus in the brain with a robust density of GABAergic axon terminals, the effect of PALDA and 2APB on Ca^{2+} entry may involve GABAergic nerve terminals too, whereas the effect of OLDA and NADA may be more restricted to the input terminals.

4.3. N-*acyldopamines depolarized striatal nerve terminals via targets other than the TRPV$_1$R*

Notably, different concentrations of the general $TRPV_1R$ agonists, capsaicin, anandamide and *R*-methanandamide did not trigger significant $[Ca^{2+}]_i$ rise, Ca^{2+} uptake and evoked transmitter release. This is in agreement with recent findings indicating that PKC activation is a pre-requisite to prevent rapid $TRPV_1R$ desensitization in striatal glutamatergic terminals (Musella et al., 2009; Maccarrone et al., 2008), which is probably also true for dopaminergic terminals in our experimental models. Yet, anandamide had a small but significant stimulatory effect on the release of dopamine. This indicates that the close to significant effect of anandamide in the Ca^{2+} assay might have resulted from the stimulation of a small Ca^{2+} entry in a subpopulation of the total nerve terminal population.

Altogether, 1) the lack of effect of capsaicin, anandamide and *R*-methanandamide; 2) the agonist activity of PALDA; 3) the lack of antagonism by iodoresiniferatoxin, SB3366791, and Ruthenium Red; and 4) the persisting effects in the $TRPV_1R$ knockout mice all suggest that *N*-acyldopamines posses additional, previously unidentified targets to control striatal input terminals.

4.4. *Possible targets of* N-*acyldopamines*

The targets of *N*-acyldopamines may be cation channels in the cell membrane. Concluding from the experiments without K^+ in the buffer and with nifedipine, these *N*-acyldopamine receptors are not sensitive to membrane potential and/or are not linked to activation of voltage-gated Ca^{2+} channels. Furthermore, they are highly sensitive to the channel blocker Gd^{3+} whereas the K^+-evoked release of the two transmitters was not.

Although 2APB is a sensitizer and activator of the $TRPV_2R$ and the $TRPV_3R$ (Hu et al., 2004), these novel *N*-acyldopamine receptors do not seem to be identical with any known member of the TRPV family: 1) we found that Gd^{3+} was a two-three orders of magnitude more potent antagonist than at the $TRPV_1R$ and the $TRPV_2R$ channels (Tousova et al., 2005; Leffler et al., 2007); and 2) Ruthenium Red, the general inhibitor of the $TRPV_{1-6}$ channels, failed to prevent the action of NADA. Additionally, anandamide and 2-AG are activators of the $TRPV_4R$, and the lack of their putative effect in the Ca^{2+} level assays do not indicate the involvement of $TRPV_4Rs$ in the observed effect of *N*-acyldopamines (Nilius et al., 2004). $TRPA_1$ receptors can be activated by WIN55212-2 (Jeske et al., 2006) which did not affect Ca^{2+} levels in our study. $TRPC_6$ and $TRPM_8$ receptors are blocked by 2APB, therefore these channels were also unlikely involved in the effect of *N*-acyldopamines (Hu et al., 2004).

Antagonists of other common ion channels such as ionotropic glutamate receptors, $GABA_A$ receptors, 5-HT_3 serotonin and nicotinic receptors as well as P2X ATP receptors failed to prevent the effect of NADA. We can also exclude the possible involvement of metabotropic cannabinoid receptors linked to Ca^{2+} rise in neurons, namely, the CB_1R and the GPR55 (Lauckner et al., 2005; Pertwee, 2007) because of the rapid kinetics of Ca^{2+} entry and the lack of effect of WIN55212-2 and 2-AG. The lack of functional CB_1Rs in striatal dopaminergic terminals (Köfalvi et al., 2005; Uchigashima et al., 2007) further argues against the involvement of CB_1Rs.

Finally, we can exclude the involvement of (TASK-like) K^+ channel blockade as a depolarizing mechanism. We have shown previously that TASK-3 K^+ channels control hippocampal nerve terminal membrane potential. TASK-1 and TASK-3 channels can be blocked either by anandamide and WIN55212-2 (Maingret et al., 2001) or by Ruthenium Red (Czirják and Enyedi, 2003). As in the striatal nerve terminals neither anandamide nor Ruthenium Red nor WIN55212-2 induced depolarization, and most importantly, the effect of NADA persists in the absence of K^+, we can exclude the involvement of TASK-1 and 3 channels.

Curiously, the $TRPV_1R$ antagonist capsazepine significantly inhibited the action of NADA on the release of dopamine. This should account for the partial inhibition of the NADA-induced $[Ca^{2+}]_i$ rise. Several present-time cation channels developed from one common ancestor, therefore there is a relatively large structural and/or sequence similarity among many ligand- and voltage-gated ion channels bringing about a substantial cross-reaction among their "selective" ligands (Köfalvi, 2008). Capsazepine has been reported to block nAChRs (Liu and Simon, 1997), HCN1 channels (Ray et al., 2003; Gill et al., 2004), and voltage-gated Ca^{2+} channels (Docherty et al., 1997). Hence, it is not difficult to imagine that if capsazepine competitively antagonizes the effect of NADA at the $TRPV_1R$ it can do so at another related ion channel.

4.5. *Possible consequences of the presynaptic action of* N-*acyldopamines*

Little is known about the recently discovered series of neurotransmitter-fatty acid conjugates such as *N*-arachidonoyl-glycin, *N*-arachidonoyl-serotonin or *N*-arachidonoyldopamine. The latter is shown to be formed and released in dopamine-rich regions (Marinelli et al., 2007) but neither its source nor its enzymatic pathways is delineated. *N*-acyldopamines may sensitize nerve terminals at subthreshold concentrations with small increases in nerve terminal Ca^{2+} levels that could amplify the size of transmitter release upon weak axonal depolarization. Alternatively, they may directly induce transmitter release whereby decreasing the signal-to-noise ratio, that is, the reliability of synaptic communication. They seem capable of inducing Ca^{2+} overload which may result in Ca^{2+}-mediated cell death. It would be also interesting to know how *N*-acyldopamine levels change in dopamine deficiency, *i.e.* in Parkinson's disease and how these changes contribute to the development of syndromes.

References

Avelino, A., Cruz, F., 2006. $TRPV_1$ (vanilloid receptor) in the urinary tract: expression, function and clinical applications. Naunyn Schmiedebergs Arch. Pharmacol. 373, 287–299.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Chu, C.J., Huang, S.M., De Petrocellis, L., Bisogno, T., Ewing, S.A., Miller, J.D., Zipkin, R.E., Daddario, N., Appendino, G., Di Marzo, V., Walker, J.M., 2003. *N*-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. J. Biol. Chem. 278, 13633–13639.

Cristino, L., de Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., Di Marzo, V., 2006. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 139, 1405–1415.

Czirják, G., Enyedi, P., 2003. Ruthenium red inhibits TASK-3 potassium channel by interconnecting glutamate 70 of the two subunits. Mol. Pharmacol. 63, 646–652.

de Lago, E., de Miguel, R., Lastres-Becker, I., Ramos, J.A., Fernández-Ruiz, J., 2004. Involvement of vanilloid-like receptors in the effects of anandamide on motor behavior and nigrostriatal dopaminergic activity: in vivo and in vitro evidence. Brain Res. 1007, 152–159.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and

structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.
Di Marzo, V., Lastres-Becker, I., Bisogno, T., De Petrocellis, L., Milone, A., Davis, J.B., Fernandez-Ruiz, J.J., 2001. Hypolocomotor effects in rats of capsaicin and two long chain capsaicin homologues. Eur. J. Pharmacol. 420, 123–131.
Docherty, R.J., Yeats, J.C., Piper, A.S., 1997. Capsazepine block of voltage-activated calcium channels in adult rat dorsal root ganglion neurones in culture. Br. J. Pharmacol. 121, 1461–1467.
Gill, C.H., Randall, A., Bates, S.A., Hill, K., Owen, D., Larkman, P.M., Cairns, W., Yusaf, S.P., Murdock, P.R., Strijbos, P.J., Powell, A.J., Benham, C.D., Davies, C.H., 2004. Characterization of the human HCN1 channel and its inhibition by capsazepine. Br. J. Pharmacol. 143, 411–421.
Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.
Hamon, B., Glowinski, J., Giaume, C., 1997. Nicotine inhibits slowly inactivating K^+ currents in rat cultured striatal neurons. Pflugers Arch. 434, 642–645.
Hu, H.Z., Gu, Q., Wang, C., Colton, C.K., Tang, J., Kinoshita-Kawada, M., Lee, L.Y., Wood, J.D., Zhu, M.X., 2004. 2-Aminoethoxydiphenyl borate is a common activator of $TRPV_1$, $TRPV_2$, and $TRPV_3$. J. Biol. Chem. 279, 35741–35748.
Huang, S.M., Bisogno, T., Trevisani, M., Al-Hayani, A., De Petrocellis, L., Fezza, F., Tognetto, M., Petros, T.J., Krey, J.F., Chu, C.J., Miller, J.D., Davies, S.N., Geppetti, P., Walker, J.M., Di Marzo, V., 2002. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc. Natl. Acad. Sci. U.S.A. 99, 8400–8405.
Jeske, N.A., Patwardhan, A.M., Gamper, N., Price, T.J., Akopian, A.N., Hargreaves, K.M., 2006. Cannabinoid WIN 55,212-2 regulates $TRPV_1$ phosphorylation in sensory neurons. J. Biol. Chem. 281, 32879–32890.
Köfalvi, A., 2008. Alternative interacting sites and novel receptors for cannabinoid ligands. In: Köfalvi, A. (Ed.), Cannabinoids and the Brain. Springer US, pp. 131–160.
Köfalvi, A., Oliveira, C.R., Cunha, R.A., 2006. Lack of evidence for functional $TRPV_1$ vanilloid receptors in rat hippocampal nerve terminals. Neurosci. Lett. 403, 151–156.
Köfalvi, A., Rodrigues, R.J., Ledent, C., Mackie, K., Vizi, E.s., Cunha, R.A., Sperlágh, B., 2005. Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. J. Neurosci. 25, 2874–2884.
Köfalvi, A., Pereira, M.F., Rebola, N., Rodrigues, R.J., Oliveira, C.R., Cunha, R.A., 2007. Anandamide and NADA bi-directionally modulate presynaptic Ca^{2+} levels and transmitter release in the hippocampus. Br. J. Pharmacol. 151, 551–563.
Lauckner, J.E., Hille, B., Mackie, K., 2005. The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB_1 receptor coupling to $G_{q/11}$ G proteins. Proc. Natl. Acad. Sci. U.S.A. 102, 19144–19149.
Leffler, A., Linte, R.M., Nau, C., Reeh, P., Babes, A., 2007. A high-threshold heat-activated channel in cultured rat dorsal root ganglion neurons resembles $TRPV_2$ and is blocked by gadolinium. Eur. J. Neurosci. 26, 12–22.
Liu, L., Simon, S.A., 1997. Capsazepine, a vanilloid receptor antagonist, inhibits nicotinic acetylcholine receptors in rat trigeminal ganglia. Neurosci. Lett. 228, 29–32.
Lovinger, D.M., 2008. Presynaptic modulation by endocannabinoids. Handb. Exp. Pharmacol. 184, 435–477.
Maccarrone, M., Rossi, S., Bari, M., De Chiara, V., Fezza, F., Musella, A., Gasperi, V., Prosperetti, C., Bernardi, G., Finazzi-Agrò, A., Cravatt, B.F., Centonze, D., 2008. Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. Nat. Neurosci. 11, 152–159.
Maingret, F., Patel, A.J., Lazdunski, M., Honoré, E., 2001. The endocannabinoid anandamide is a direct and selective blocker of the background K^+ channel TASK-1. EMBO J. 20, 47–54.
Marinelli, S., Di Marzo, V., Berretta, N., Matias, I., Maccarrone, M., Bernardi, G., Mercuri, N.B., 2003. Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat substantia nigra by endogenous stimulation of vanilloid receptors. J. Neurosci. 23, 3136–3144.
Marinelli, S., Di Marzo, V., Florenzano, F., Fezza, F., Viscomi, M.T., van der Stelt, M., Bernardi, G., Molinari, M., Maccarrone, M., Mercuri, N.B., 2007. *N*-arachidonoyl-dopamine tunes synaptic transmission onto dopaminergic neurons by activating both cannabinoid and vanilloid receptors. Neuropsychopharmacology 32, 298–308.
Marinelli, S., Pascucci, T., Bernardi, G., Puglisi-Allegra, S., Mercuri, N.B., 2005. Activation of $TRPV_1$ in the VTA excites dopaminergic neurons and increases chemical- and noxious-induced dopamine release in the nucleus accumbens. Neuropsychopharmacology 30, 864–870.
Mezey, E., Tóth, Z.E., Cortright, D.N., Arzubi, M.K., Krause, J.E., Elde, R., Guo, A., Blumberg, P.M., Szallasi, A., 2000. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc. Natl. Acad. Sci. U.S.A. 97, 3655–3660.
Musella, A., De Chiara, V., Rossi, S., Prosperetti, Ch., Bernardi, G., Maccarrone, M., Centonze, D., 2009. TRPV1 channels facilitate glutamate transmission in the striatum. Mol. Cell. Neurosci. 40, 89–97.
Nagy, I., White, J.P.M., Paule, C.C., Maze, M., Urban, L., 2008. Functional molecular biology of the $TRPV_1$ ion channel. In: Köfalvi, A. (Ed.), Cannabinoids and the Brain. Springer US, pp. 101–130.
Nilius, B., Vriens, J., Prenen, J., Droogmans, G., Voets, T., 2004. $TRPV_4$ calcium entry channel: a paradigm for gating diversity. Am. J. Physiol. Cell Physiol. 286, 195–205.
Pertwee, R.G., 2007. GPR55: a new member of the cannabinoid receptor clan? Br. J. Pharmacol. 152, 984–986.
Raiteri, L., Raiteri, M., 2000. Synaptosomes still viable after 25 years of superfusion. Neurochem. Res. 25, 1265–1274.
Ray, A.M., Benham, C.D., Roberts, J.C., Gill, C.H., Lanneau, C., Gitterman, D.P., Harries, M., Davis, J.B., Davies, C.H., 2003. Capsazepine protects against neuronal injury caused by oxygen glucose deprivation by inhibiting I_h. J. Neurosci. 23, 10146–10153.
Roberts, J.C., Davis, J.B., Benham, C.D., 2004. [^{3}H]Resiniferatoxin autoradiography in the CNS of wild-type and TRPV1 null mice defines TRPV1 (VR-1) protein distribution. Brain Res. 995, 176–183.
Salminen, O., Murphy, K.L., McIntosh, J.M., Drago, J., Marks, M.J., Collins, A.C., Grady, S.R., 2004. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. Mol. Pharmacol. 65, 1526–1535.
Sanchez, J.F., Krause, J.E., Cortright, D.N., 2001. The distribution and regulation of vanilloid receptor VR1 and VR1 5′ splice variant RNA expression in rat. Neuroscience 107, 373–381.
Starowicz, K., Nigam, S., Di Marzo, V., 2007. Biochemistry and pharmacology of endovanilloids. Pharmacol. Ther. 114, 13–33.
Stella, N., Schweitzer, P., Piomelli, D., 1997. A second endogenous cannabinoid that modulates long-term potentiation. Nature 388, 773–778.
Szabó, T., Bíró, T., Gonzalez, A.F., Palkovits, M., Blumberg, P.M., 2002. Pharmacological characterization of vanilloid receptor located in the brain. Brain Res. Mol. Brain Res. 98, 51–57.
Tóth, A., Boczán, J., Kedei, N., Lizanecz, E., Bagi, Z., Papp, Z., Édes, I., Csiba, L., Blumberg, P.M., 2005. Expression and distribution of vanilloid receptor 1 ($TRPV_1$) in the adult rat brain. Brain Res. Mol. Brain Res. 135, 162–168.
Tousova, K., Vyklicky, L., Susankova, K., Benedikt, J., Vlanchova, V., 2005. Gadolinium activates and sensitizes the vanilloid receptor $TRPV_1$ through the external protonation site. Mol. Cell. Neurosci. 30, 207–217.
Tzavara, E.T., Li, D.L., Moutsimilli, L., Bisogno, T., Di Marzo, V., Phebus, L.A., Nomikos, G.G., Giros, B., 2006. Endocannabinoids activate transient receptor potential vanilloid 1 receptors to reduce hyperdopaminergia-related hyperactivity: therapeutic implications. Biol. Psychiatry 59, 508–515.
Uchigashima, M., Narushima, M., Fukaya, M., Katona, I., Kano, M., Watanabe, M., 2007. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J. Neurosci. 27, 3663–3676.
Zhou, F.M., Wilson, C.J., Dani, J.A., 2002. Cholinergic interneuron characteristics and nicotinic properties in the striatum. J. Neurobiol. 53, 590–605.