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doi: 10.1016/j.neuint.2007.06.023

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Abstract

β-NAD^+ is as abundant as ATP in neuronal cells. β-NAD^+ functions not only as a coenzyme but also as a substrate. β-NAD^+-utilizing enzymes are involved in signal transduction. We focus on ADP-ribosyl cyclase/CD38 which synthesizes cyclic ADP-ribose (cADPR), a universal Ca^{2+} mobilizer from intracellular stores, from β-NAD^+. cADPR acts through activation/modulation of ryanodine receptor Ca^{2+} releasing Ca^{2+} channels. cADPR synthesis in neuronal cells is stimulated or modulated *via* different pathways and various factors. Subtype-specific coupling of various neurotransmitter receptors with ADP-ribosyl cyclase confirms the involvement of the enzyme in signal transduction in neurons and glial cells. Moreover, cADPR/CD38 is critical in oxytocin release from the hypothalamic cell dendrites and nerve terminals in the posterior pituitary. Therefore, it is possible that pharmacological manipulation of intracellular cADPR levels through ADP-ribosyl cyclase activity or synthetic cADPR analogues may provide new therapeutic opportunities for treatment of neurodevelopmental disorders.

Key words: β-NAD^+, cADP-ribose, ADP-ribosyl cyclase, CD38, oxytocin, autism, pharmacological intervention.

1. Introduction

β-NAD^+ is a well-known coenzyme serving as a major acceptor of hydrogen ion in numerous metabolic reactions. The redox state of the β-NAD^+ pool appears to be an important control parameter in metabolic regulation, DNA replication and repair, transcription, resistance to oxidative stress. In neuronal cells β-NAD+ has been proposed to act as synaptic modulator (Snell et al., 1984). Understanding of the role of β-NAD^+ in cellular processes has been expanded by the demonstration of enzymatic conversion of β-NAD^+ into various products acting as key regulators of DNA stability, protein functioning, ion homeostasis and cellular activities and also disease processes (Belenky et al., 2007).

Basal levels of β-NAD^+ in cells are submillimolar levels but are different in various types of cells: 70 pmol/10^6 lymphocytes (Berger et al., 1982), 1000-1500 pmol/10^6 3T3 cells (Jacobson and Jacobson, 1976), 4 nmol/10^6 neuroblastoma x glioma hybrid NG108-15 cells (Higashida et al., 1995), and are very much affected by the state of proliferation, differentiation, or functional activity.

Using high performance liquid chromatography fraction analysis, it is established that numerous smooth muscle preparations, including the canine mesenteric artery and vein, release beta-nicotinamide adenine dinucleotide upon short-pulse electrical field stimulation in tetrodotoxin- and omega-conotoxin GVIA-sensitive manners from postganglionic nerve terminals (Smyth 2006a).

2. ADP-ribosyl cyclase

Enzymes able to catalyze hydrolysis of β-N-glycosidic bond in the molecule of β-NAD+ *in vivo* belong to the family of NAD^+-glycohydrolases (EC 3.2.2.6). CD38 represents one example of these enzymes. It possesses the capability to catalyze different reactions, such as the hydrolysis of β-NAD^+ and cyclic ADP-ribose (cADPR) to ADP-ribose but also the cyclization of β-NAD+ and nicotinamine guanine dinucleotide (NGD^+) to cADPR and cGDPR, respectively (Magni et al., 2004). Biological activity of

cADPR is well defined recently, as described below. Furthermore, CD38 catalyzes a base exchange reaction between NADP+ and nicotinic acid, thereby producing nicotinamide and nicotinate adenine dinucleotide phosphate ($NAADP^+$), another powerful Ca^{2+} mobilizer (Fliegert et al., 2007).

Originally, enzymes catalyzing the synthesis of cADPR have been known for many years as NAD^+ glycohydrolases that cleave β-NAD^+ to ADP-ribose and nicotinamide. The first enzyme identified as ADP-ribosyl cyclase was isolated from the mollusk *Aplysia californica, as a* 30 kDa soluble protein containing 258 amino acid residues (Lee, 2001). It was cloned and exhibited sequence similarity to the human antigens CD38 and BST1/BP3. A sequence of 19 amino acids in the middle of the protein is highly conserved among two different species of *Aplysia* as well as in CD38 from human, mouse, and rat. The main difference in the catalytic activity of *Aplysia* protein and CD38 is attributed to their ability to hydrolase cADPR (*Aplysi*a cyclase can not do that), and to the amount of formed cADPR very small in a case of CD38 (the overall reaction is the same as for classical NAD^+ glycohydrolases), and predominant in a case of *Aplysia* enzyme. Therefore, strictly speaking, the term NAD^+ glycohydrolase refers to an enzymatic reaction, and distinct enzymes like ADP-ribosyl transferases and poly(ADP-ribose)polymerases possess NAD^+-glycohydrolase activity. Thus ADP-ribosyl cyclases are a functionally and structurally defined class of enzymes which are not similar to the definition of NAD^+ glycohydrolases (Lee et al., 1999). However, more recently, *Aplysia* ADP-ribosyl cyclase was shown to be cADPR hydrolase, but hydrolase activity becomes detectable only at very high enzyme concentrations and may have no physiological meaning (Cakir-Kiefer et al., 2000).

Unfortunately, little is known about regulation of ADP-ribosyl cyclase or CD38 expression in neuronal cells. Bruzzone *et al.* (2004) have reported glutamate-mediated CD38 overexpression in astrocytes, thereby suggesting the role of CD38 in neuronal-glia communication. Furthermore, molecular mechanism on developmental regulation of CD38 in the central nervous system (CNS) has not been reported.

Analysis of the distribution of human CD38 in normal tissues revealed its abundant expression in various cells including cells of neuronal origin. Interestingly, no fetal organ or tissue ever expressed CD38 (Fernandez et al., 1998). However, in the CNS,

ADP-ribosyl cyclase activity corresponding to CD38 was detected as early as embryonic day 15 of mice development (Ceni et al., 2003), and the activity enhanced further starting from postnatal days (our unpublished data).

The catalytic domain of ADP-ribosyl cyclase has been reported to reside extracellularly on the membranes of majority of the cells tested including rat cortical astrocytes (Pawlikowska et al., 1996). Predominant intracellular localization (or both types of localization) of ADP-ribosyl cyclase has been detected in some cells such as cortical astrocytes (Hotta et al., 2000) and neuroblastoma x glioma NG108-15 hybrid cells (Higashida et al., 2001a). The intracellular cADPR levels appear to be at picomolar (mg protein) concentrations in various tissues (Welseth et al., 1991; Higashida et al., 1997a, 1999, 2000b and 2007). Intracellular cyclase activity was shown to be associated with cytosolic fraction, mitochondrial membrane, nuclear membrane, endoplasmic reticulum (Matsumura and Tanuma, 1998; Brailoiu and Miyamoto, 2000). The specific features of ADP-ribosyl cyclase expressed in various cell compartments are not clear yet.

In murine brain, CD38 was found in both neurons and glial cells, showing predominant intracellular location, and was enriched in neuronal perikarya (Ceni et al., 2003; Jin et al., 2007). In human brain, CD38 immunoreactivity was demonstrated in the perikarya and dendritis of many neurons (Mizuguchi et al., 1995). In rat astrocytes, ADPribosyl cyclase has been reported to have both intracellular and extracellular actions (Hotta et al., 2000). Moreover, co-culture of astrocytes with neurons resulted in the significant overexpression of astrocyte CD38 both on the plasma membrane and intracellularly, and this effect was attributed to neuron-released glutamate action on astrocytes (Bruzzone et al., 2004).

Taking into account predominant extracellular position of CD38, the mechanism of its action has been proposed. It includes two possible scenarios: (1) catabolism of extracellular β-NAD^+ (persisted there permanently or released from damaged cells) followed by the channeling of cADPR through the hydrophilic cavity within the cyclase molecule (De Flora et al., 2004); (2) endocytosis of CD38 and its functioning to produce cADPR intracellularly (Zocchi et al., 1999). Franco et al. (1998) have provided the evidence that CD38 is a channel for transmembrane influx of cADPR: the highly cationic cADPR could potentially associate with the anionic head groups of

membrane phospholipids, allowing the central cavity of CD38 to form a hydrophilic channel for its transport.

Recently, it has been reported that extracellular application of cADPR induced oxytocin release in posterior pituitary nerve endings, while no release was found in the same preparations isolated from CD38 knockout mice, probably because without CD38 cADPR was not entered into the inside (Jin et al., 2007). This is supported by the following observation, in that the preparation was once treated with digitonin to permeabilized, extracellular cADPR elicited oxytocin release in CD38 knockout preparations (Jin et al., 2007). Similarly, in astrocytes, extracellular application of NAADP can induce Ca^{2+} signaling (Heidemann et al., 2005). There are observations that when CD38 is expressed in CD38KO cells, it is driven to the plasma membrane, endoplasmic reticulum, and nuclear membrane without any free enzyme in the cytosol. Though precise mechanisms of direction of CD38 to intracellular membranes remain to be unclear, probably, the molecule has amino-terminal signal for targeting (Sun et al., 2002).

Recently, we reported the brain regional distribution of ADP-ribosyl cyclase activity in mice. Wild-type mice showed the highest cyclase activity in the hypothalamus, followed by the cerebellum, cerebrum and posterior pituitary (Jin et al., 2007) (Fig. 1). This was matched by high levels of mRNA and immunoreactivity in the hypothalamus. Along with the loss of CD38 expression, brain tissues from CD38KO mice showed a marked reduction of ADP-ribosyl cyclase activity and mRNA levels. The cADPR content in the hypothalamus was correspondingly reduced in the hypothalamus and neurohypophysis in CD38KO mice.

Colocalization of CD38 with tyrosine hydroxylase in perivascular autonomic nerve terminals was characterized (Smyth et al., 2006b), suggesting new mechanisms of the β-NAD^+/CD38 system in autonomic neurovascular signaling.

3. Sites of action of cADPR

cADPR specifically binds to calcium-storing microsome from sea urchin eggs (Lee, 1991), thus directly binds to type II and III ryanodine receptors to initiate its function of releasing Ca^{2+} (Meszaros et al., 1993). In cADPR-treated heart muscle, the

number of Ca^{2+} sparks fluorimetrically observed increases (Lukyanenko and Gyorke, 1999), indicating that cADPR increases the frequency of Ca^{2+} release. Type III ryanodine receptors of bovine and mouse skeltal muscle cells show that the open probability is increased by 20-fold and the affinity to Ca^{2+} increases by 10-fold (from 0.38 to 3.2 mM) in the presence of 1 mM cADPR (Sonnleitner et al., 1998). The role of cADP-ribose as a potent Ca^{2+} mobilizer has been described (Cancela et al., 2000; Higashida et al., 2001b, 2003). As a result, enhanced acetylcholine release from frog motor nerve terminals has been shown by cADPR- and ryanodine-dependent Ca^{2+} increases (Hachikusa et al., 2007).

On the other hand, there is growing evidence indicating that cADPR may function through its specific receptor proteins, a 100 kDa protein (Walseth et al., 1993) and/or FKBP12.6 (Noguchi et al., 1997), the binding proteins for FK506, an immunosupressant drug. FKBP12.6 binds to type II ryanodine receptors. It is believed that dissociation from ryanodine receptors activates or delays inactivation of them (Ahern et al., 1997). Upon FK506 addition, Ca^{2+} is released from type II ryanodine receptors. Pretreatment of ryanodine receptors with FK506 decreases cADPR-induced Ca^{2+} release, suggesting that cADPR and FK506 interact at the same site on FKBP12.6 (Noguchi et al., 1997). Therefore, the possibility exists that cADPR functions on type II ryanodine receptors indirectly through FKBP12.6 as well as by direct binding to ryanodine receptors. Calmodulin is also an activator of Ca^{2+} release from ryanodine receptors (Lee et al., 1994). Phosphorylation by Ca^{2+}-Calmodulin-dependent protein kinase also involves in upregulation of ryanodine receptors (Takasawa et al., 1995).

Although many ryanodine-sensitive processes are known in the nervous system, very little information directly relating to the function of cADPR at the pharmacological and molecular level has been reported. Hashii *et al.* (2000) have reported that FK506 efficiently inhibits depolarization induced $[Ca^{2+}]_i$ increases in NG108-15 cells. Furthermore, FKBP12.6 has yet to be detected in the nervous system, thereby rendering it difficult to determine the exact site of action of cADPR in neuronal cells. Regarding FKBP12, its expression in neurons is up-regulated by cerebral ischemia (Brecht et al., 2003). FKBP12 declined in dying neurons, whereas FKBP12 was upregulated in less severely injured neurons, thus suggesting that FKBP12 plays an important role in the

process of neuronal survival and death following cerebral ischemia, and is involved in inflammatory reactions that occur within an area of infarction (Kato et al., 2000).

There is a plenty of evidence that cADPR is a second messenger in Ca^{2+} signaling in the nervous system. Differences in the response pattern of $[Ca^{2+}]_i$ have permitted the division of the cADPR effect in neurons, glial cells or neuronal tumor cells induced by the injection or addition of cADPR into three groups: 1) no immediate $[Ca^{2+}]_i$ increase; 2) $[Ca^{2+}]_i$ increases can be elicits soon after the infusion of cADPR through patch pippettes; 3) potentiation of $[Ca^{2+}]_i$ increases primed by depolarization (Higashida et al., 2001a).

Recently, the role of ADPR in the regulation of Kv7 (KCNQ2/3) M-type potassium channels (Higashida et al., 1995 and 2000a) and nonspecific cation channels activity (TRPM2) has been described in activated microglial cells and heterologously expressed HEK cells. It was suggested that β-NAD^+ could be converted to ADPR by enzymatic activity of β-NAD^+ glycohydrolases, such as the membrane-bound enzyme CD38 (Kraft et al., 2004). Therefore, CD38, in addition to other β-NAD^+ utilizing enzymes, may function in some cells of the CNS for producing ADP-ribose, while cADPR might be as side product of this reaction.

4. Regulation of cADPR production

The formation of cADPR is enhanced by nitric oxide (NO) and cyclic GMP (cGMP) in homogenates of sea urchin eggs (Galione, 1993). Since NO and cGMP are formed in response to hormones and neurotransmitters, the cADPR level may be indirectly controlled by receptors, through a long cascade that culminates in activation of cytosolic ADP-ribosyl cyclase by cGMP-dependent protein phosphorylation (Clementi et al., 1996; Willmott et al., 1996). The cGMP-stimulated ADP-ribosyl cyclase process has been proved in rat hippocampal slice. Ca^{2+} released from presynaptic and postsynaptic intracellular stores plays important roles in activity-dependent synaptic plasticity, including long-term depression (LTD) of synaptic strength. Presynaptic ryanodine receptor-gated stores appear to mobilizesome of the Ca^{2+} necessary to induce LTD. Generation of intracellular messenger NO in postsynaptic pyramidal neurons

stimulates production of cyclic GMP which activates PKG. The latter causes stumulation of cADP-ribose synthesis, which enhance RyR-mediated Ca^{2+} release necessary to induce LTD (Reyes-Harde et al., 1999).

Control of the membrane-bound form of ADP-ribosyl cyclase was first reported in NG108-15 cells by Higashida *et al.* (1997a and b). They demonstrated that stimulation of muscarinic mAChRs with agonists activates or inhibits cADPR formation in mAChR-overexpressing NG108-15 hybrid cells in a subtype-dependent fashion. Interestingly, activation of ADP-ribosyl cyclase by carbamylcholine at a relatively low concentration was observed in membranes obtained from NG108-15 cells independently expressing m1 or m3 mAChRs, while inhibition was mediated by endogenous m4 mAChRs and exogenous m2 mAChRs. M1-mAChR-mediated increase in cADPR formation was proved in the intact tissue, rat superior cervical ganglion (Zhang et al., 2005).

The most critical experiments for determining cADPR as a second messenger downstream of m1 mAChRs are to measure agonist stimulated changes in intracellular NAD+ concentrations. It was shown that intracellular β-NAD^+ levels were reduced transiently after ACh stimulation in m1 AChRs in NG108-15 cells (Higashida et al., 1997a), whose time course resembles that of phosphatidyl inositol-4,5-bisphosphate breakdown stimulated by bradykinin in the same cell line. This result is consistent with that NAD^+ is consumed for producing cADPR as a substrate. The mirror time course of production of cADPR and substrate consumption of β-NAD inside the NG108-15 cells was demonstrated after challenging with bradykinin (Higashida et al., 2006). Fig. 2 is the first demonstration of the substrate-metabolite change expected in ADP-ribosyl cyclase action.

Above-mentioned effects in NG108-15 cells were mimicked by submicromolar levels of GTP. The ACh-induced activation was inhibited by prior treatment of cells with cholera toxin, while the inhibition of ADP-ribosyl cyclase was sensitive to pertussis toxin, which enabled us to hypothesize that the signal to ADP-ribosyl cyclase from mAChRs is mediated through different G proteins (Higashida, 1997b). This signaling appears to be comparable to other G-ptotein coupled receptors expressed in NG108-15 cells (Noda et al., 1996), namely the αB2 adrenergic, δ opioid,

B2 bradykinin and P2u ATP receptors, which have been shown to activate or inhibit adenylyl cyclase or to stimulate phospholipase Cb *via* G proteins. Nearly identical regulation is found in the isoproterenoland GTP-induced increase in [^{3}H]cADPR or cyclic guanosine diphosphate-ribose formation in crude membranes of cortical astrocytes (Hotta et al., 2000). This increase can be blocked by the prior treatment of cells with cholera toxin, but not with pertussis toxin, suggesting that the signal of β-adrenergic stimulation is transduced to membrane-bound ADP-ribosyl cyclase *via* G proteins within the surface membranes of astrocytes.

The action of cADPR in dorsal root ganglion neurons resulting in Ca^{2+} release from intracellular stores appears to be similar to metabotropic receptor activation, suggesting additional receptor-operated pathway for controlling Ca^{2+} response in neuronal cells (Crawford et al., 1997; Pollock et al., 1999). Activation of 5-HT5A serotonin receptors in neurons leads to the inhibition of ADP-ribosyl cyclase activity, thus suggesting competitive mechanism in regulation of cADPR production in neuronal cells expressing both 5-HT5A receptors and neurotransmitter receptors whose stimulation results in activation of the enzyme (Noda et al., 2003).

In chromaffin cells, stimulation with cholinergic agonists, excessive KCl depolarization and 8-bromo-cyclic AMP induces activation of ADP-ribosyl cyclase activity in membranes (Morita et al., 1997). This activation is dependent on Ca^{2+} influx and subsequent cAMP-dependent protein kinase A (A-kinase) activation, suggesting that A-kinase-dependent phosphorylation of membrane ADP-ribosyl cyclase is involved. However, such molecular mechanisms have not been examined in normal brain cells. But, recently, A-kinase dependent regulation of ADP-ribosyl cyclase has been reported in adult cardiomyocytes, in which possibly a new species of cyclase of 42 kDa functions (Xie et al., 2003).

In airway smooth muscle cells, expression of CD38 is regulated by IL-13, a cytokine implicated in the pathogenesis of asthma. The intracellular calcium responses to bradykinin, thrombin, and histamine are significantly higher in cells treated with IL-13 suggesting involvement of cADPR dependent pathway in the modulation of calcium signaling (Deshpande et al., 2005). In pancreatic acinar cells, hormonal stimulation with acetylcholine, cholecystokinin, or a membrane-permeant analog of cGMP increased ADP-ribosyl cyclase activity in the cytosol (Sternfeld et al., 2003). In general, muscarinic

Ca^{2+} signaling in pancreatic cells involves cADPR-dependent mechanisms as it was shown in CD38 knockout mice (Fukushi et al., 2001).

Angiotensin II (Ang II) increased ADP-ribosyl cyclase activity in ventricular muscle of neonatal rats (Higashida et al., 2000b). Stimulation of ADP-ribosyl cyclase was reproduced by GTP and GTP-γ-S, and was prevented by GDP-β-S. Prior treatment of rats with cholera toxin also blocked the Ang II-induced activation. Ang II receptors detected as [^{3}H]Ang II binding were higher in density in neonatal than adult rats. These results demonstrate the existence of a signaling pathway from Ang II receptors to membrane-bound ADP-ribosyl cyclase in the ventricular muscle cell, and suggest that the Ang II-induced increase in cADP-ribose synthesis is involved in regulation of cardiac function and development after birth, during which the wet weight of rat hearts increases. Thus, a role of cADPR in transcriptional upregulation through NFAT3 in rat hearts at the neonatal period is proposed, and this proposal is based on the hypothesis for cardiac hypertrophy induced by Ang II, in which calcineurin-dependent dephosphorylation of NFAT3 is critical and FK506 or cyclosporin A can inhibits hypertrophy (Molkentin et al., 1998). cADPR system appears to play a signalling role in BK induced aldosterone synthesis in the human adrenal gland, where significantly increased activity of ADP-ribosyl cyclase was detected upon the action of Ang II (Takeda et al., 2005). However, the role of Ang II on ADP-ribosyl cyclase in the CNS is unanswered.

In murine N9 microglial cells, lipopolysaccharide induced microglia-activating pathway, i.e. expression of inducible nitric oxide synthase, overproduction and release of nitric oxide and of tumor necrosis factor alpha. In lipopolysaccharide-stimulated murine N9 microglial cells, cyclic ADP-ribose was increased by 1.7-fold with activation of cyclase, paralleled by doubling of the basal [Ca^{2+}]i levels (Franco et al., 2006). The identification of cyclic ADP-ribose as a key signal metabolite in the complex cascade of events triggered by lipopolysaccharide.

5. Discovery of the role of CD38 in the hypothalamus and posterior pituitary

Higashida and his colleagues have recently shown that CD38 is required in mice for the regulation of social behavior, including pair cognition and nurturing

behaviors by regulating oxytocin, originally known as the hormone for female's uterine contraction during labor and mild ejection in lactation to babies (Jin et al., 2007).

CD38 knocked out (KO) female mice displayed disrupted maternal behavior after separation of a mother and pups. We observed dam's behavior with their newborn pups in home cages in which pups were temporarily placed outside the nest. Normal dams retrieved their babies precisely and very quickly to the same small area of the nest. CD38KO dams took a long time to start to retrieve and behaved as if they were not interested in their babies or 'neglected' pups. They often dropped them during retrieving on the way to the nest, as if they do not remember their nest and so that pups were scattered in different places. However, the CD38KO mothers fed the pups sufficiently well for them to grow to the same weight as the control pups. These results show the clear mother's abnormal nurturing behavior in CD38KO postpartum mice under a stressful condition, such as separation.

Next, we examined in CD38KO male mice, which experienced repeated pairings with the same female mouse. Wild-type mice showed a significant decline in the time spent investigating a female upon subsequent presentations of the same female. This phenotype is due to not loosing interest to the pair but to what they memorized the paired female. So they do no need to further investigate, but instantaneously they recognize the pair. In contrast, CD38KO males showed sustained high levels of investigation at each encounter with the same female and the same level of investigation, in another words, they behave as a 'stoker'.

Since CD38KO mice had no deficits in either olfactory-guided foraging or habituation to a non-social olfactory stimulus as tested by the preference ratio of consumption of isovaleric acid solution in their drinking water, the impairment of social memory did not depend on deficits in main olfactory bulb function. Although the function of CD38 in social behavior could be very specific to the particular neural circuitry involved, the data so far do not rule out a more general cognitive dysfunction. CD38 mutants could learn the shock experience in the passive avoidance test. We concluded that CD38KO males with persistent interest during repeated presentations fail to develop their social memory.

The above abnormality resembles a memory deficit observed in oxytocin gene or oxytocin receptor knockout mice, in which oxytocin is not produced or even if

produced, oxytocin has no effect. To link between CD38 and oxytocin, we measured plasma and cerebrospinal fluid oxytocin levels and found that, CD38KO mice had reduced oxytocin levels, but elevated levels in the hypothalamus and pituitary in the brain. This indicates, although oxytocin is produced and packaged into vesicles in the hypothalamic neurons and posterior pituitary nerve endings in CD38KO mice, it was not released into the brain and blood stream. Therefore, oxytocin less functions. Indeed, the behavioral phenotype of CD38KO mice could be normalized even by subcutaneous oxytocin injections. We also tried a genetic approach by infusion of a lentivirus carrying the human CD38 gene into the third ventricle of knockout mice. This procedure resulted in normalization of the plasma and CSF oxytocin level and thereby of rescued social memory, indicating that the mechanisms underlying social behavior require CD38-dependent oxytocin secretion.

In conclusion, our findings shed light on the function of CD38 in the brain and in human diseases associated with abnormal social behavior such as developmental disorder, including autism. Most interestingly, our result can give a theoretical basis for one immediately-applicable treatment to a subset of autism patients by oxytocin administration, but not by gene therapy of expressing CD38, which takes a long way.

6. Conclusion

We reviewed recent studies that elucidated the role of cADPR in neurons, glial cells, neuroendocrine cells and neuronal tumor cells. There are accumulating evidence for the important role of cADPR in the CNS function. Especially, the recent finding in cADPR/CD38-dependent hormone secretion in the hypothalamus is remarkable (Fig. 3). It may provide a new way to elucidate etiology of neurodevelopmental disorders. However, many fundamental questions about the cADPR signal pathway in the brain remain unanswered.

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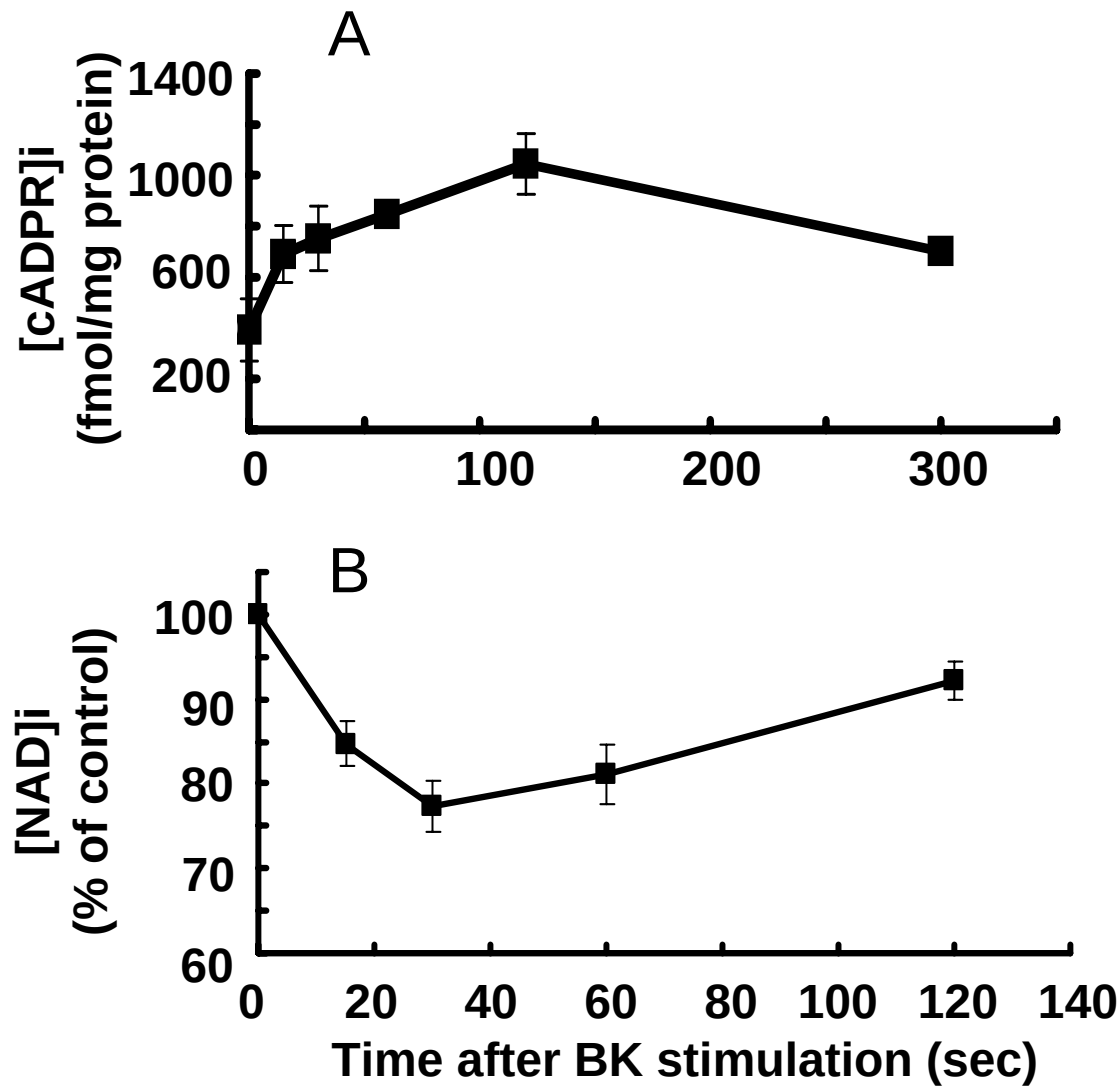
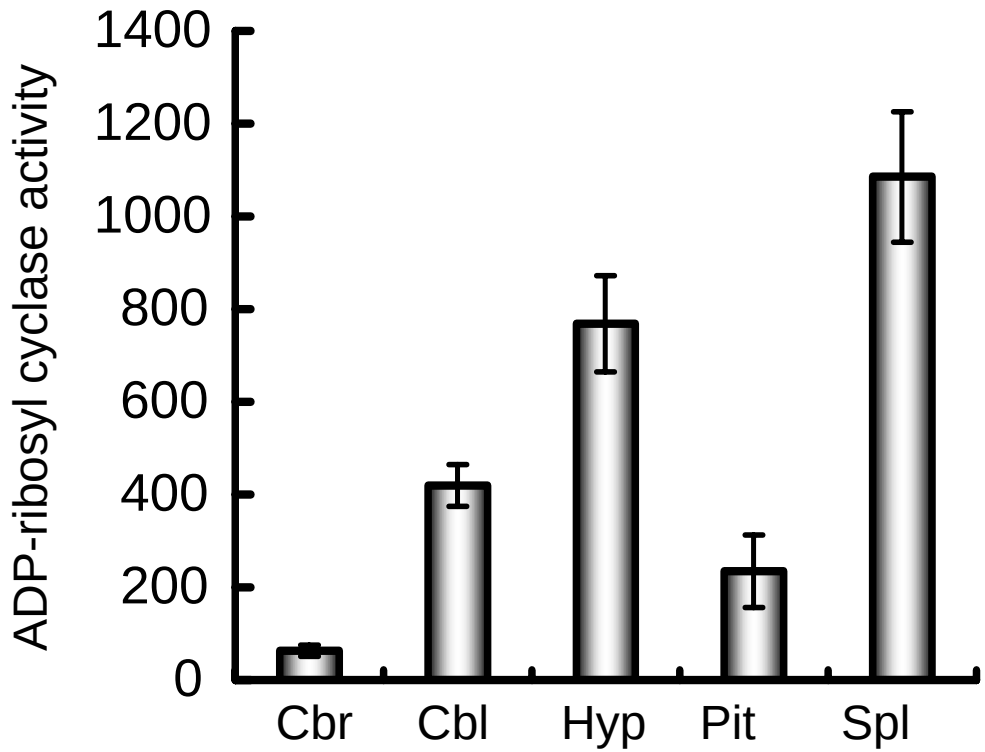
Figure legends

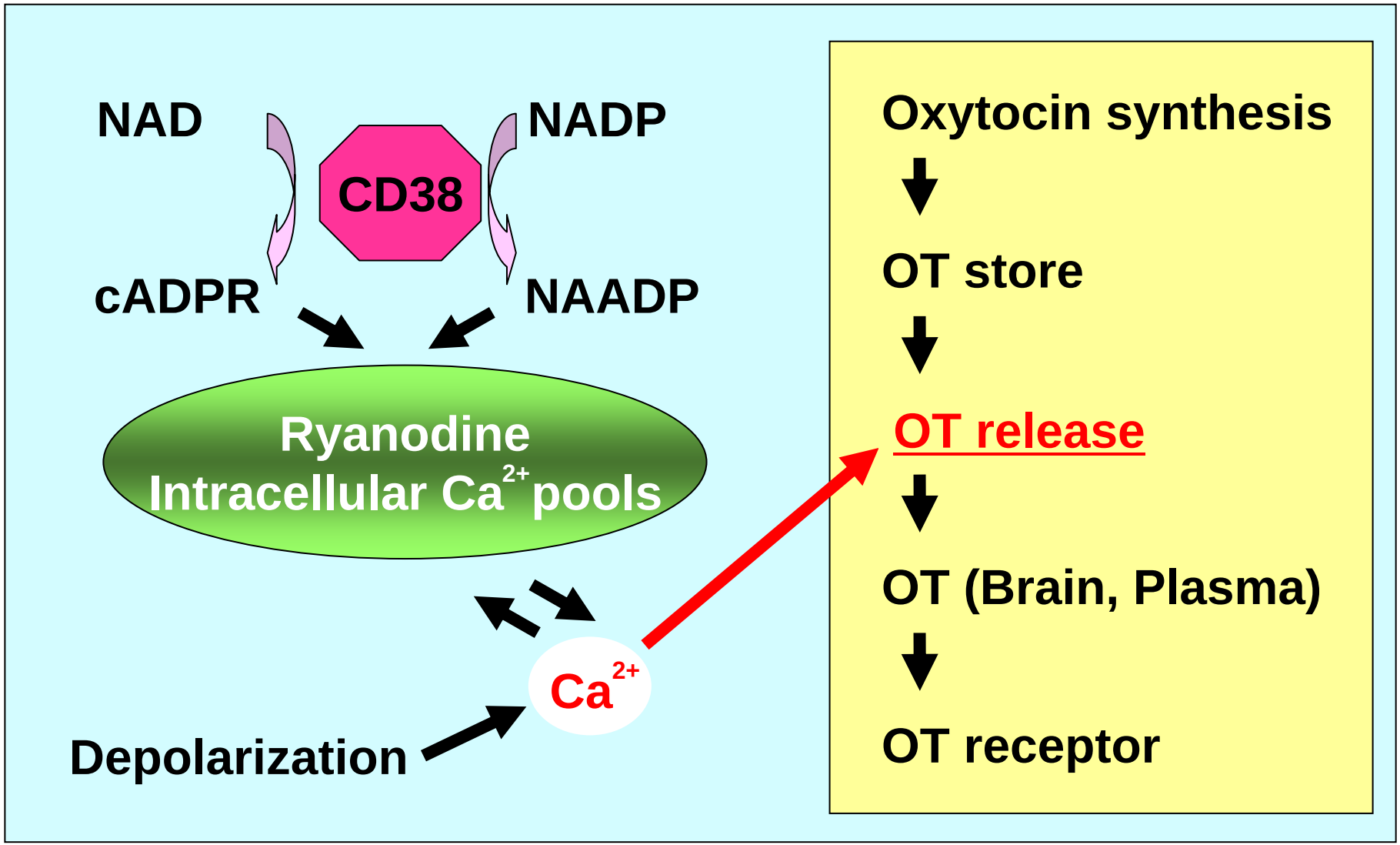
Fig. 1. ADP-ribosyl cyclase activity. ADP-ribosyl cyclase activity, measured as the rate of cyclic GDP-ribose formation by whole cell homogenates isolated from various tissues of wild-type mice. Unit=pmol/min/mg protein. N=4-12. Cbr, cerebrum; Cbl, cerebellum; Hyp, hypothalamus; Pit, posterior pituitary; Spl, spleen. (Modified from supplementary Figure S11c of Jin et al. (2007).

Fig. 2. Bradykinin-induced changes in intracellular concentrations of cADPR and NAD in neuronal NGPM1-27 cells. Live NGPM1-27 neuroblastoma x glioma hybrid cells overexpressing muscarinic M1 receptors and expressing endogenous bradykinin (BK) B2 receptors were cultured. Bradykinin (BK) was applied at time zero. (A) Time course of intracellular concentrations of cADPR ([cADPR]i). (B) Time course of intracellular concentrations of NAD+ ([NAD+]i). The control value for the NAD+ level in NGPM1-27 cells was 4.9±0.4 nmol/10^6 cells. Values represent the means±S.E.M of three dishes in triplicate cultures. (Modified from Fig. 2 of Higashida et al., 2006).

Fig. 3. A scheme showing that CD38-dependent cADPR- and NAADP-sensitive intracellular Ca^{2+} mobilization from ryanodine receptors in microsomes has a key role in oxytocin (OT) release. See more detail in Jin et al., 2007.


A
[cADPR]i
(fmol/mg protein)
1400
1000
600
200
0
100
200
300
B
[NAD]i
(% of control)
100
90
80
70
60
0
20
40
60
80
100
120
140
Time after BK stimulation (sec)


ADP-ribosyl cyclase activity
1400
1200
1000
800
600
400
200
0
Cbr
Cbl
Hyp
Pit
Spl


NAD
NADP
CD38
cADPR
NAADP
Ryanodine
Intracellular Ca2+ pools
Ca2+
Depolarization
Oxytocin synthesis
OT store
OT release
OT (Brain, Plasma)
OT receptor