Amphetamine Sensitization Elevates CaMKII β mRNA

RACHEL GREENSTEIN, GABRIELA NOVAK,* AND PHILIP SEEMAN

Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

KEY WORDS schizophrenia; gene expression; dopamine supersensitivity; CaMKII α ; CaMKII β ; CaMK2

ABSTRACT Recent studies have shown that the elevation in calcium/calmodulin-dependent protein kinase II (CaMKII) may play an important role in amphetamine-induced dopamine release, as well as in the increase of dopamine D2 receptor high-affinity states in psychosis. Because amphetamine sensitization is a widely used animal model of psychosis or schizophrenia, we investigated whether amphetamine sensitization results in an overall increase in the α and β subunits of CaMKII. To answer this question, we measured CaMKII α and β subunit mRNA expression using Real-Time Quantitative PCR in amphetamine-sensitized rat striata, compared to saline-treated controls. The results were then standardized to β -glucuronidase, a housekeeping gene. Our results showed a statistically significant increase in the CaMKII β subunit, and an increase in the α subunit which did not reach statistical significance. Because the levels of both CaMKII β and CaMKII α play a role in neuronal function and synapse formation, the present finding of an elevated level of CaMKII β and α subunit mRNA in the amphetamine-sensitized model of psychosis points to the possibility of dysregulated levels of CaMKII subunits in human psychosis. **Synapse 61:827–834, 2007.**

© 2007 Wiley-Liss, Inc.

INTRODUCTION

Although many genes are associated with schizophrenia (Glatt and Jonsson, 2006; Jonsson et al., 2003; Lewis et al., 2003), no single gene has emerged with major effect. Animal models of psychosis, therefore, may be helpful in identifying susceptibility genes (Ko et al., 2006; Seeman et al., 2006a; Seeman et al., 2005b). One such animal model is amphetamine sensitization. This model mimics several biological aspects of schizophrenia (Seeman et al., 2002), including dopamine supersensitivity, a common feature in schizophrenia (Seeman et al., 2007).

Dopamine supersensitivity is associated not only with elevated levels of D2^{High} receptors [i.e., dopamine D2 receptors in their state of high-affinity for dopamine; (Seeman et al., 2005b; Seeman et al., 2006)], but also with an enhanced release of dopamine (Seeman et al., 2005b). In turn, enhanced dopamine release due to sensitization depends on calmodulin (CaM), Ca²⁺, and on the activity of CaMKII, and is independent of vesicular dopamine storage (Iwata et al., 1997). Amphetamine-induced dopamine release in sensitized animals is mediated by CaMKII-enhanced outward transport of dopamine rather than by exocytosis, distinguishing it from dopamine release

in nontreated rats or after acute administration of amphetamine (Kantor et al., 1999).

Since CaMKII has a central role in amphetamine sensitization, it likely plays an important role in the development of schizophrenia. CaMKII is a neuronal signaling protein that comprises 1–2% of the protein in the brain (Kolodziej et al., 2000) and is highly enriched in the postsynaptic density fraction (Peng et al., 2004). It is a serine/threonine protein kinase, which modulates synaptic plasticity and plays a role in neuronal development, maturation, and signaling (Fukunaga and Miyamoto, 1999). CaMKII is an evolutionarily conserved protein and is encoded by four genes (α , β , γ , and δ) (Tobimatsu and Fujisawa, 1989). Most CaMKII holoenzymes are 12–14 subunit α/β

Contract grant sponsors: the Essel Foundation, Constance Lieber, Stephen Lieber, the National Alliance for Research on Schizophrenia and Depression (NARSAD), the Stanley Medical Research Institute (SMRI), the Canadian Institutes for Health Research (CIHR), the Ontario Mental Health Foundation (OMHF), the National Institute on Drug Abuse (NIDA).

*Correspondence to: Gabriela Novak, Department of Pharmacology, University of Toronto, Medical Sciences Building 4344, 1 King's College Circle, Toronto, Canada M5S 1A8. E-mail: gabriela.novak@utoronto.ca

Received 28 December 2006; Accepted 12 April 2007

DOI 10.1002/syn.20429

Published online in Wiley InterScience (www.interscience.wiley.com).

heteromers (Rosenberg et al., 2006). The ratio of the subunits depends on the type, activity, and the developmental stage of the neuron (Brocke et al., 1999). This ratio also regulates the translocation time of the α/β heteromer from F-actin to the postsynaptic density where it phosphorylates its targets (Shen and Meyer, 1999) and also determines the sensitivity of the CaMKII holoenzyme to Ca^{2+} signals (Brocke et al., 1999). The two subunits differ in their sensitivity to calcium signals. The α subunit is sensitive to signals involving high levels of Ca^{2+} , while the β subunit is sensitive to low level calcium signals (Brocke et al., 1999). When Ca^{2+} enters through NMDA receptors in the dendrites, it causes upregulation of the expression of the α subunit. The β subunit, on the other hand, is increased in response to the blockade of AMPA receptors, leading to an increased presence of CaMKII at the postsynaptic density (Thiagarajan et al., 2002). Even in the absence of a sustained elevation in calcium, CaMKII can stay active for a prolonged period of time because of autophosphorylation, forming a molecular memory of calcium signals in the neuron (Miller et al., 1988). CaMKII also regulates the availability and release of synaptic vesicles at the presynaptic terminals by interaction with various proteins; for example, CaMKII phosphorylates synapsin I and promotes dissociation of synapsin I from synaptic vesicles, making more vesicles available for neurotransmitter release (Greengard et al., 1993). In addition, by phosphorylating the N-terminus of dopamine transporter (DAT), CaMKII α plays a key role in mediating the ability of amphetamine to induce efflux of dopamine through the DAT (Fog et al., 2006).

Because the β subunit was found to be significantly elevated in postmortem frontal cortex tissues of patients exhibiting schizophrenia and depression (Novak et al., 2006), and because the α and β isoforms are important in neurodevelopment, neural function and in mediating amphetamine sensitization, we have measured the levels of the α and β subunits in striata from rats sensitized to amphetamine.

MATERIALS AND METHODS

Animals

We used Sprague Dawley male rats, weighing ~200 g at the start of the experiments. The rats were randomly divided into two groups, one group ($n = 8$) received an intraperitoneal injection of d-amphetamine sulphate in a volume of 0.5 mg/1 ml after dissolving in 0.9% NaCl, and the second group ($n = 6$) received an intraperitoneal injection of saline. The injections were given on Monday, Wednesday, and Friday for 3 weeks. The amphetamine-treated rats received increasing doses of amphetamine for a total of 9 injections over the 3 weeks period. The dose increased from 1 to 3 mg/kg with an increase of 1 mg/

kg each week. Following the last injection, the animals were left drug-free for 26 days before they were euthanized by carbon dioxide overdose. The entire striatum tissue was dissected without subdivision into sections in order to provide sufficient tissue for extraction of total RNA. The weighted average for each tissue was between 20–25 mg.

The withdrawal period induced an intense behavioural sensitization in these animals (Seeman et al., 2002). The brain striata were removed and frozen at -70°C until used.

Extraction of total RNA from tissues

Seven hundred fifty microliter of TRIzol reagent (Invitrogen; Life Technologies, Carlsbad, CA) was added to the tissue, according to the manufacturer's guidelines, to extract total RNA from tissues. (Plytron PT-10 probe, Brinkmann instruments, Westbury, NY). The tissue was homogenized as a whole with no separation between the postsynaptic and the presynaptic regions because of the small size of the striatum and the requirement for a large amount of RNA for determination of RNA quantity. The homogenized samples were incubated at room temperature for 5 min, 200 μl chloroform were added, and the tubes were shaken vigorously for 15 s. The tubes were incubated at room temperature for 3 min and centrifuged at 10,000 rpm at 4°C for 15 min. The aqueous upper phase containing RNA was transferred to a new tube. The RNA was washed with 1 volume of 70% EtOH/DEPC'd water, and transferred immediately onto a Qiagen RNeasy Mini Spin Column. The samples were centrifuged for 15 s at 10,000 rpm. To remove contaminants, the columns were washed with 350 μl of buffer RW1 and centrifuged for 15 s at 11,000 rpm. Residual DNA was removed by adding DNase1 incubation mix (10 μl of DNase1 and 70 μl buffer RDD) directly onto the RNeasy silica-gel membrane and incubating it at room temperature for 15 min. The columns were washed again with 350 μl RW1 buffer and centrifuged for 15 s at 11,000 rpm. To remove any additional contamination, we washed the columns twice with 500 μl of RPE buffer and centrifuged, first for 15 s at 11,000 rpm, and the second time for 2 min at 11,000 rpm to dry the silica-gel membrane.

To elute the RNA, we pipetted 40 μl of RNase-free water directly onto the RNeasy silica-gel membrane, let it stand for 1 min, and then centrifuged it for 1 min at 11,000 rpm. We repeated the elution with another 20 μl of RNase-free water and centrifuged the membrane for another min. The final total RNA concentration was between 750–1000 ng per μl of water, as determined by optical density. This solution was further diluted with DEPC water to 3 μg RNA per 8 μl water for cDNA synthesis.

First strand cDNA synthesis

The SUPERScript III^{TM} First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) was used to prepare cDNA, using oligo (dT)₂₀ and 3 μ g of total RNA. The reaction was terminated by incubation at 70°C for 15 min and the sample was then chilled on ice for 5 min. The RNA was removed by adding 1 μ l of RNase H and incubating at 37°C for 20 min. The resulting sample containing 21 μ l cDNA was stored at -20°C for later use.

Real-time quantitative polymerase chain reaction

The real-time PCR Mx4000^{TM} Multiplex Quantitative PCR System (Stratagene, La Jolla, CA) was used to quantitate and analyze cDNA. Specific primers were designed for each target gene by using the Primer Express software (Perkin-Elmer Applied Biosystems, Foster City, CA) and synthesized by SIGMA-Genosys (Oakville, Ontario). A standard template was prepared for each target gene by conventional PCR using Taq DNA Polymerase (QIAGEN, Mississauga, ON). We used the same primer pairs which were later used in the real-time quantitative PCR. We confirmed that the PCR product obtained is the correct target gene by verifying the correct size of the fragment on an agarose gel after amplification of the template. The PCR products were extracted from a gel using the MinElute^{TM} Gel Extraction Kit (Qiagen, Mississauga, ON) and the concentration was measured by optical density. We used Brilliant^{TM} SYBR® Green QPCR Core Reagent Kit (Stratagene, La Jolla, CA) to prepare the reaction mixture for the real-time quantitative PCR, the company protocol was followed. The total reaction volume of 25 μ l contained 0.25 μ l (10 mM) of forward and 0.25 μ l (10 mM) of reverse primer. For each set of primers a dissociation curve analysis was performed to verify that only a single product is amplified. The β -glucuronidase forward primer (5' to 3') was AGAGGTTACGGTTCAGTGC and the reverse primer (5' to 3') was TCCCCCTTG TGTGTTCA. The CAMKII α forward primer (5' to 3') was AGGATGAAGACACCAAAGTGC and the reverse primer (5' to 3') was GGTTCAAAGGCTGTC ATTCC. The CAMKII β forward primer (5' to 3') was ACCTCATTTGAGCCTGAAGC and the reverse primer (5' to 3') was CAGGATAGTGGTGTGGATCG. SYBR Green dye was diluted 1:2000 and the Rox reference dye was diluted 1:500. 3 μ l of 1/10 diluted cDNA sample (equivalent to 43 ng of total RNA) was used per reaction. The cycling parameters were 95°C for 10 min to activate the SureStart^{TM} Taq polymerase, followed by 40 cycles of denaturation at 95°C for 30 s, reannealing at 55°C (for CAMKII α), 54°C (for CAMKII β) and 60°C (for β -glucuronidase) for 45 s, and extension at 72°C for 45 s. To account for the

varying RNA quality between individual samples and the differences in efficiency in cDNA synthesis of different cDNA batches, the mRNA expression of each target gene was normalized against the mRNA expression of a house keeping gene, β -glucuronidase. In order to minimize the effect of background noise and well to well variation on the results observed, our data were normalized against the Rox reference dye and the algorithm of adaptive baseline. Each reaction was repeated at least four times, using two independent cDNA dilutions and each of the fourteen RNA samples is represented by three independent cDNA syntheses.

RESULTS

Before proceeding with cDNA measurements, we confirmed that the rat tissues were indeed supersensitive to dopamine by measuring their proportion of D2^{High} receptors in two representative striata from the amphetamine treated group, a reliable index of dopamine supersensitivity (Seeman et al., 2005b; Seeman et al., 2007). We measured the levels of D2^{High} receptors using a range of concentrations of [³H](+)-PHNO D2 agonist, which has a high affinity for the dopamine D2^{High} receptors, in the presence and absence of guanine nucleotide. Guanine nucleotide converts all the D2^{High} receptors into their state of low affinity for dopamine; however, because [³H](+)-PHNO binds also to the low-affinity state of dopamine D2 receptors, we measured the total amount of [³H](+)-PHNO bound to D2 receptors and the amount of bound [³H](+)-PHNO which was sensitive to the presence of guanine nucleotide. The last measurement reflected the component of the D2^{High} receptors. (Seeman et al., 2007). Indeed, the striata from the amphetamine-sensitized rats revealed that the proportion of dopamine D2^{High} receptors was 2.2-fold higher than the control tissues (data not shown), confirming previously published data (Seeman et al., 2005b; Seeman et al., 2007).

We measured the cDNA concentration of CaMKII α and CaMKII β using real-time quantitative PCR. We normalized it against the expression of β -glucuronidase, a house keeping gene, to reduce the effects of mRNA manipulation, such as variations in cDNA synthesis and mRNA degradation. (Novak et al., 2006). To determine the significance of changes in mRNA levels, we used the Student *t*-distribution test (TDIST), a two tailed-test.

Expression of CaMKII α mRNA

The average concentration of CaMKII α mRNA in the striata of control rats was 3.0 $\pm$ 0.2 pg mRNA/ μ g total RNA (mean $\pm$ SE; *n* = 6). The average CaMKII α mRNA concentration in the amphetamine sensitized group was 3.3 $\pm$ 0.1 pg mRNA/ μ g total RNA (mean $\pm$ SE; *n* = 8), a 10% increase (TDIST; *P* = 0.03) (Fig. 1).

Fig. 1. CaMKII α in amphetamine sensitized rat striatum. The amount of CaMKII α transcript in the control striata and in the amphetamine sensitized tissues. The average was 3.0 ± 0.2 pg mRNA/ μ g total RNA (mean $\pm$ SE; $n = 6$), and 3.3 ± 0.1 pg mRNA/ μ g total RNA (mean $\pm$ SE; $n = 8$), respectively. This was a 10% increase (TDIST; $P = 0.03$).

Expression of CaMKII β mRNA

The average concentration of CaMKII β mRNA in the striata of the control rats was 3.5 ± 0.1 pg mRNA/ μ g total RNA (mean $\pm$ SE; $n = 6$). The average CaMKII β mRNA concentration in the amphetamine-sensitized group was 4.0 ± 0.2 pg mRNA/ μ g total RNA (mean $\pm$ SE; $n = 8$), a 15% increase (TDIST; $P = 0.01$) (Fig. 2).

Expression of β -glucuronidase

The average concentration of β -glucuronidase mRNA in the striata of the control rats was 30 ± 1 fg mRNA/ μ g total RNA (mean $\pm$ SE; $n = 6$). The average concentration of β -glucuronidase mRNA in the striata of the amphetamine sensitized group was 32 ± 1 fg mRNA/ μ g

Fig. 2. CaMKII β in amphetamine sensitized rat striatum. The amount of CaMKII β mRNA transcript in the control striata and in the amphetamine sensitized tissues. The average concentration of CaMKII β mRNA in the striata of the control rats was 3.5 ± 0.1 pg mRNA/ μ g total RNA (mean $\pm$ SE; $n = 6$) and in the amphetamine sensitized group it was 4.0 ± 0.2 pg mRNA/ μ g total RNA (mean $\pm$ SE; $n = 8$), a 15% increase (TDIST; $P = 0.01$).

total RNA (mean $\pm$ SE; $n = 8$). There was no statistically significant difference between the two groups.

Normalization of CaMKII α and CaMKII β to β -glucuronidase

CaMKII mRNA levels of each cDNA sample were standardized to the housekeeping gene β -glucuronidase by dividing the CaMKII α or CaMKII β concentration by the concentration of β -glucuronidase. The average concentration of CaMKII α in the control rats after the normalization was 98 ± 5 pg CaMKII α /pg β -glucuronidase (mean $\pm$ SE; $n = 6$). The average concentration of CaMKII α in the amphetamine-sensitized rats after the normalization was 103 ± 4 pg CaMKII α /pg β -glucuronidase (mean $\pm$ SE; $n = 8$).

Fig. 3. CaMKII α / β -glucuronidase in amphetamine sensitized rat striata. The amount of CaMKII α transcript in the control rat striata and in the sensitized tissues after standardization to β -glucuronidase. The average is 98 ± 5 pg CaMKII α /pg β -glucuronidase (mean $\pm$ SE; $n = 6$) and 103 ± 4 pg CaMKII α /pg β -glucuronidase (mean $\pm$ SE; $n = 8$), respectively. The 6% increase in CaMKII α was not statistically significant (TSIDT; $P = 0.2$).

The 6% increase in CaMKII α was not statistically significant (TSIDT; $P = 0.2$) (Fig. 3).

The average concentration of CaMKII β in the control rats after the normalization was 116 ± 3 pg CaMKII β /pg β -glucuronidase (mean $\pm$ SE; $n = 6$). The average concentration of CaMKII β in the amphetamine-sensitized rats after the normalization was 125 ± 4 pg CaMKII β /pg β -glucuronidase (mean $\pm$ SE; $n = 8$). The 8% increase in CaMKII β was statistically significant (TDIST; $P = 0.02$) (Fig. 4).

DISCUSSION

The present results confirm that dopamine supersensitivity and the increase in the high-affinity states

Fig. 4. CaMKII β / β -glucuronidase in amphetamine sensitized rat striata. The amount of CaMKII β transcript in the control rat striata and in the sensitized tissues after standardization to β -glucuronidase. The average is 116 ± 3 pg CaMKII β /pg β -glucuronidase (mean $\pm$ SE; $n = 6$) and 125 ± 4 pg CaMKII β /pg β -glucuronidase (mean $\pm$ SE; $n = 8$), respectively. This 8% increase in CaMKII β is statistically significant (TDIST; $P = 0.02$).

of dopamine D2 receptors are associated with an elevation in both CaMKII β and CaMKII α ; however, only CaMKII β elevation reaches statistical significance. These findings support the involvement of CaMKII, especially CaMKII β , in schizophrenia, as well as in amphetamine sensitization (Novak et al., 2006).

CaMKII plays an important role in mediating responses to changes in synaptic activity that lead to neuronal development, maturation, and signaling (Fukunaga and Miyamoto, 1999); therefore, any change in the concentration of CaMKII α and β subunits would have a major effect on the neuronal activity. The precise maintenance of the α and β subunit ratio is essential because it is directly related to neural

maturation, determining under which conditions the synapse will undergo either LTP or LTD (Fukunaga and Miyamoto, 1999). Consequently, CaMKII α knock-out mice show defects in expression of LTP, LTD, and in spatial learning (Silva et al., 1992).

The concentration of CaMKII α and β subunits is critical in neurodevelopment, especially during the period of synaptic network formation, because the concentrations of both CaMKII subunits in the brain changes in a specific pattern during the postnatal period. In the early stages of development the concentration of CaMKII α is low, but it increases steadily and seems to be limited to cells that are no longer dividing (Bayer et al., 1999; Brocke et al., 1995). Compared to the α subunit, β expression begins early in development, rises until day 3 after birth, when expression reaches the adult level, and continues to be expressed strongly in the adult rat brain (Bayer et al., 1999; Fink et al., 2003; Karls et al., 1992). The α : β ratio changes throughout development. In the forebrain of a 10-day old rat, the ratio between α and β is 1:1, compared with an adult, where the ratio is 2.3:1 (Kelly et al., 1987; McGuinness et al., 1985; Miller and Kennedy, 1985). Increased CaMKII levels, especially of CaMKII β , which are observed in schizophrenia patients and in amphetamine sensitized rats, may play a role in abnormal neurodevelopment and subsequent predisposition to schizophrenia (Novak et al., 2006). In addition, absence of maturation, induced by CaMKII upregulation, leads to neuropathology and behavioral defects in adulthood (Keshavan et al., 1994).

The α and β subunits hold very different functions; β controls dendritic morphology and the number of synapses and synaptic formation, whereas α controls synaptic strength (Fink et al., 2003). This plays an important role not only during development, but also in the mature brain. The ratio between α and β subunits is inversely regulated in response to activity, the α subunit levels rising during higher firing levels and β subunit levels increasing during lower activity. The result is reinforcement of existing synapses during high firing activity and formation of new ones during lower activity (Fink et al., 2003; Thiagarajan et al., 2002).

The role of the β subunit in the α / β hetero-oligomer is to dock the holoenzyme to polymerized F-actin and upon stimulation to translocate the whole functional unit to the postsynaptic density (PSD), its main site of action. A small number of the β subunits is able to localize a much larger number of the α subunits to synaptic and cytoskeletal sites, most importantly the PSD (Shen et al., 1998). In contrast, α homo-oligomers remain localized to soma and dendrites (Blichenberg et al., 2001; Mayford et al., 1996; Merrill et al., 2005). Hence, even a small change in CaMKII β levels has the potential to result in a large effect on CaMKII concentration at the PSD, which is its main site

of action, and, consequently, on the activity of the neuron.

Schizophrenia is a disorder with structural and functional abnormalities distributed in the thalamo-cortico-limbic regions of the brain. Hypofunction of a subpopulation of cortico-limbic NMDA receptors may participate in this pathophysiology (Tsai and Coyle, 2002). Noncompetitive antagonists of the NMDA receptor, such as phencyclidine, ketamine, and MK 801 can cause psychotic-like cognitive impairment (Javitt and Zukin, 1991; Krystal et al., 1994; Krystal et al., 2000). The PSD contains diverse types of ionotropic glutamate receptors such as NMDA, AMPA, and metabotropic receptors, all of which are substrates for CaMKII (Yoshimura et al., 2002). CaMKII not only greatly influences their function (Leonard et al., 1999; Swope et al., 1999), but also influences their developmental expression (Ben-Ari et al., 1997). In addition, NMDA receptor activity influences the expression of the α subunit, and AMPA receptors influence the expression of the β subunit of CaMKII (Thiagarajan et al., 2002). In striatal neurons both dopamine and glutamate receptors are densely expressed and CaMKII is the most potent kinase that regulates NR2B phosphorylation (Liu et al., 2006). Chronic intermittent stimulation of striatal dopaminergic receptors enhances the sensitivity of NMDA receptors in the striata (Kotter, 1994). The D2 receptor mediates phosphorylation of NR2B subunit by activation of CaMKII and subsequently affects glutamatergic transmission (Oh et al., 1999). Liu, et al., showed that acute cocaine administration reduced NMDA receptor phosphorylation by CaMKII through a D2 receptor-dependent mechanism (Liu et al., 2006). They observed that in response to cocaine administration, D2 receptors directly interact with the NR2B subunit and disturb the NR2B-CaMKII binding, subsequently preventing NR2B phosphorylation by CaMKII. In addition, the interruption of the interaction between the dopamine-glutamate signaling led to disrupting of various forms of synaptic and behavioral plasticity in response to stimulants (Liu et al., 2006). Accordingly, we assume that amphetamine sensitization and concomitant DAT phosphorylation and D2 receptor stimulation may escalate the disruption between NMDA receptor and CaMKII, affecting CaMKII α levels and upregulating CaMKII β , resulting in increase in AMPA receptors at the PSD and a decrease in NMDA receptors, leading to NMDA hypofunction. When we consider the NMDA hypofunction theory, one should keep in mind that the noncompetitive antagonists of the NMDA receptor have a combined action on both dopamine and NMDA receptors and might act as dopamine partial agonists. Furthermore, glutamate receptor agonists induce a very mild improvement in symptoms when used in the treatment of psychosis (Seeman et al., 2005a).

Other evidence of a significant role for CaMKII in neuron function is its ability to phosphorylate tyrosine hydroxylase and tryptophan hydroxylase (Yamauchi, 2005), because the production of monoamines is important in psychiatric illnesses.

To conclude, CaMKII is important for the normal function of D2, NMDA, and AMPA receptors. Any alteration in the concentration of its α and β subunits would result in impairment in neuronal development, maturation, and signaling. The data show that dopamine supersensitivity is associated with a statistically significant elevation in the β subunit mRNA levels, but not the α subunit mRNA levels of CaMKII. Since the elevation of the CaMKII subunit mRNA level was relatively low, we would suggest for future research to examine different brain regions, such as the hippocampus and the frontal cortex.

ACKNOWLEDGMENTS

The authors thank the Medland family, the O'Rorke family, the Rockert family, and the estate of the late Dr. Karolina Jus for their support. Supported by the Canadian Institutes for Health Research, the Ontario Mental Health Foundation, the Essel Foundation, and by Constance E. Lieber and Stephen Lieber.

REFERENCES

- Bayer KU, Lohler J, Schulman H, Harbers K. 1999. Developmental expression of the CaM kinase II isoforms: Ubiquitous γ - and δ -CaM kinase II are the early isoforms and most abundant in the developing nervous system. *Brain Res Mol Brain Res* 70:147–154.
- Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa JL. 1997. GABAA, NMDA and AMPA receptors: A developmentally regulated 'menage a trois'. *Trends Neurosci* 20:523–529.
- Blichenberg A, Rehbein M, Muller R, Garner CC, Richter D, Kindler S. 2001. Identification of a cis-acting dendritic targeting element in the mRNA encoding the α subunit of Ca^{2+} /calmodulin-dependent protein kinase II. *Eur J Neurosci* 13:1881–1888.
- Brocke L, Srinivasan M, Schulman H. 1995. Developmental and regional expression of multifunctional Ca^{2+} /calmodulin-dependent protein kinase isoforms in rat brain. *J Neurosci* 15:6797–6808.
- Brocke L, Chiang LW, Wagner PD, Schulman H. 1999. Functional implications of the subunit composition of neuronal CaM kinase II. *J Biol Chem* 274:22713–22722.
- Fink CC, Bayer KU, Myers JW, Ferrell JE, Jr, Schulman H, Meyer T. 2003. Selective regulation of neurite extension and synapse formation by the β but not the α isoform of CaMKII. *Neuron* 39:283–297.
- Fog JU, Khoshbouei H, Holy M, Owens WA, Vaegter CB, Sen N, Nikandrova Y, Bowton E, McMahon DG, Colbran RJ, Daws LC, Sitte HH, Javitch JA, Galli A, Gether U. 2006. Calmodulin kinase II interacts with the dopamine transporter C terminus to regulate amphetamine-induced reverse transport. *Neuron* 51:417–429.
- Fukunaga K, Miyamoto E. 1999. Current studies on a working model of CaM kinase II in hippocampal long-term potentiation and memory. *Jpn J Pharmacol* 79:7–15.
- Glatt SJ, Jonsson EG. 2006. The Cys allele of the DRD2 Ser311Cys polymorphism has a dominant effect on risk for schizophrenia: evidence from fixed- and random-effects meta-analyses. *Am J Med Genet B: Neuropsychiatr Genet* 141:149–154.
- Greengard P, Valtorta F, Czernik AJ, Benfenati F. 1993. Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science* 259:780–785.
- Iwata SI, Hewlett GH, Ferrell ST, Kantor L, Gnegy ME. 1997. Enhanced dopamine release and phosphorylation of synapsin I and neuromodulin in striatal synaptosomes after repeated amphetamine. *J Pharmacol Exp Ther* 283:1445–1452.
- Javitt DC, Zukin SR. 1991. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* 148:1301–1308.
- Jonsson EG, Sillen A, Vares M, Ekholm B, Terenius L, Sedvall GC. 2003. Dopamine D2 receptor gene Ser311Cys variant and schizophrenia: Association study and meta-analysis. *Am J Med Genet B: Neuropsychiatr Genet* 119:28–34.
- Kantor L, Hewlett GH, Gnegy ME. 1999. Enhanced amphetamine- and K^{+} -mediated dopamine release in rat striatum after repeated amphetamine: Differential requirements for Ca^{2+} - and calmodulin-dependent phosphorylation and synaptic vesicles. *J Neurosci* 19:3801–3808.
- Karls U, Muller U, Gilbert DJ, Copeland NG, Jenkins NA, Harbers K. 1992. Structure, expression, and chromosome location of the gene for the β subunit of brain-specific Ca^{2+} /calmodulin-dependent protein kinase II identified by transgene integration in an embryonic lethal mouse mutant. *Mol Cell Biol* 12:3644–3652.
- Kelly PT, Shields S, Conway K, Yip R, Burgin K. 1987. Developmental changes in calmodulin-kinase II activity at brain synaptic junctions: Alterations in holoenzyme composition. *J Neurochem* 49:1927–1940.
- Keshavan MS, Anderson S, Pettegrew JW. 1994. Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex? The Feinberg hypothesis revisited. *J Psychiatr Res* 28:239–265.
- Ko F, Tallerico T, Seeman P. 2006. Antipsychotic pathway genes with expression altered in opposite direction by antipsychotics and amphetamine. *Synapse* 60:141–151.
- Kolodziej SJ, Hudmon A, Waxham MN, Stoops JK. 2000. Three-dimensional reconstructions of calcium/calmodulin-dependent (CaM) kinase II α and truncated CaM kinase II α reveal a unique organization for its structural core and functional domains. *J Biol Chem* 275:14354–14359.
- Kotter R. 1994. Postsynaptic integration of glutamatergic and dopaminergic signals in the striatum. *Prog Neurobiol* 44:163–196.
- Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr, Charney DS. 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* 51:199–214.
- Krystal JH, Bennett A, Abi-Saab D, Belger A, Karper LP, D'Souza DC, Lipschitz D, Abi-Dargham A, Charney DS. 2000. Dissociation of ketamine effects on rule acquisition and rule implementation: Possible relevance to NMDA receptor contributions to executive cognitive functions. *Biol Psychiatry* 47:137–143.
- Leonard AS, Lim IA, Hemsworth DE, Horne MC, Hell JW. 1999. Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA* 96:3239–3244.
- Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, Williams NM, Schwab SG, Pulver AE, Faraone SV, Brzustowicz LM, Kaufmann CA, Garver DL, Gurling HM, Lindholm E, Coon H, Moises HW, Byerley W, Shaw SH, Mesen A, Sherrington R, O'Neill FA, Walsh D, Kendler KS, Ekelund J, Paunio T, Lonnqvist J, Peltonen L, O'Donovan MC, Owen MJ, Wildenauer DB, Maier W, Nestadt G, Blouin JL, Antonarakis SE, Mowry BJ, Silverman JM, Crowe RR, Cloninger CR, Tsuang MT, Malaspina D, Harkavy-Friedman JM, Svrakic DM, Bassett AS, Holcomb J, Kalsi G, McQuillin A, Brynjolfsson J, Sigmundsson T, Petursson H, Jazin E, Zoega T, Helgason T. 2003. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73:34–48.
- Liu XY, Chu XP, Mao LM, Wang M, Lan HX, Li MH, Zhang GC, Parekar NK, Fibuch EE, Haines M, Neve KA, Liu F, Xiong ZG, Wang JQ. 2006. Modulation of D2R-NR2B interactions in response to cocaine. *Neuron* 52:897–909.
- Mayford M, Baranes D, Podsypanina K, Kandel ER. 1996. The 3'-untranslated region of CaMKII α is a cis-acting signal for the localization and translation of mRNA in dendrites. *Proc Natl Acad Sci USA* 93:13250–13255.
- McGuinness TL, Lai Y, Greengard P. 1985. Ca^{2+} /calmodulin-dependent protein kinase II. Isozymic forms from rat forebrain and cerebellum. *J Biol Chem* 260:1696–1704.
- Merrill MA, Chen Y, Strack S, Hell JW. 2005. Activity-driven postsynaptic translocation of CaMKII. *Trends Pharmacol Sci* 26:645–653.
- Miller SG, Kennedy MB. 1985. Distinct forebrain and cerebellar isoforms of type II Ca^{2+} /calmodulin-dependent protein kinase associate differently with the postsynaptic density fraction. *J Biol Chem* 260:9039–9046.
- Miller SG, Patton BL, Kennedy MB. 1988. Sequences of autophosphorylation sites in neuronal type II CaM kinase that control Ca^{2+} -independent activity. *Neuron* 1:593–604.
- Novak G, Seeman P, Tallerico T. 2006. Increased expression of calcium/calmodulin-dependent protein kinase II β in frontal cortex in schizophrenia and depression. *Synapse* 59:61–68.

- Oh JD, Vaughan CL, Chase TN. 1999. Effect of dopamine denervation and dopamine agonist administration on serine phosphorylation of striatal NMDA receptor subunits. *Brain Res* 821:433–442.
- Peng J, Kim MJ, Cheng D, Duong DM, Gygi SP, Sheng M. 2004. Semi-quantitative proteomic analysis of rat forebrain postsynaptic density fractions by mass spectrometry. *J Biol Chem* 279:21003–21011.
- Rosenberg OS, Deindl S, Comolli LR, Hoelz A, Downing KH, Nairn AC, Kuriyan J. 2006. Oligomerization states of the association domain and the holoenzyme of Ca^{2+} /CaM kinase II. *FEBS J* 273:682–694.
- Seeman P, Tallerico T, Ko F, Tenn C, Kapur S. 2002. Amphetamine-sensitized animals show a marked increase in dopamine D2 high receptors occupied by endogenous dopamine, even in the absence of acute challenges. *Synapse* 46:235–239.
- Seeman P, Ko F, Tallerico T. 2005a. Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. *Mol Psychiatry* 10:877–883.
- Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, Premont RT, Sotnikova TD, Boksa P, El-Ghundi M, O'Dowd BF, George SR, Perreault ML, Mannisto PT, Robinson S, Palmiter RD, Tallerico T. 2005b. Dopamine supersensitivity correlates with D2^{High} states, implying many paths to psychosis. *Proc Natl Acad Sci USA* 102:3513–3518.
- Seeman P, Schwarz J, Chen JF, Szechtman H, Perreault M, McKnight GS, Roder JC, Quirion R, Boksa P, Srivastava LK, Yanai K, Weinshenker D, Sumiyoshi T. 2006. Psychosis pathways converge via D2^{High} dopamine receptors. *Synapse* 60:319–346.
- Seeman P, McCormick PN, Kapur S. 2007. Increased dopamine D2(High) receptors in amphetamine-sensitized rats, measured by the agonist [(3H)(+)-PHNO]. *Synapse* 61:263–267.
- Shen K, Meyer T. 1999. Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. *Science* 284:162–166.
- Shen K, Teruel MN, Subramanian K, Meyer T. 1998. CaMKII β functions as an F-actin targeting module that localizes CaMKII α/β heterooligomers to dendritic spines. *Neuron* 21:593–606.
- Silva AJ, Paylor R, Wehner JM, Tonegawa S. 1992. Impaired spatial learning in α -calcium-calmodulin kinase II mutant mice. *Science* 257:206–211.
- Swope SL, Moss SI, Raymond LA, Huganir RL. 1999. Regulation of ligand-gated ion channels by protein phosphorylation. *Adv Second Messenger Phosphoprotein Res* 33:49–78.
- Thiagarajan TC, Piedras-Renteria ES, Tsien RW. 2002. α - and β CaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron* 36:1103–1114.
- Tobimatsu T, Fujisawa H. 1989. Tissue-specific expression of four types of rat calmodulin-dependent protein kinase II mRNAs. *J Biol Chem* 264:17907–17912.
- Tsai G, Coyle JT. 2002. Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol* 42:165–179.
- Yamauchi T. 2005. Neuronal Ca^{2+} /calmodulin-dependent protein kinase II—Discovery, progress in a quarter of a century, and perspective: Implication for learning and memory. *Biol Pharm Bull* 28:1342–1354.
- Yoshimura Y, Shinkawa T, Taoka M, Kobayashi K, Isobe T, Yamauchi T. 2002. Identification of protein substrates of Ca^{2+} /calmodulin-dependent protein kinase II in the postsynaptic density by protein sequencing and mass spectrometry. *Biochem Biophys Res Commun* 290:948–954.