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REGULATION OF [^{3}H]NOREPINEPHRINE RELEASE BY *N*-METHYL-D-ASPARTATE RECEPTORS IN MINISLICES FROM THE DENTATE GYRUS AND THE CA_1-CA_3 AREA OF THE RAT HIPPOCAMPUS

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Abstract—It has been reported previously that *N*-methyl-D-aspartic acid induces a significant release of [^{3}H]norepinephrine preaccumulated in slices from the hippocampus. In the present study, we investigated whether there are regional differences in the hippocampus regarding this *N*-methyl-D-aspartate effect. In the absence of Mg^{2+}, *N*-methyl-D-aspartate (10–200 μM) induced the release of [^{3}H]norepinephrine from superfused minislices containing the dentate gyrus area or the CA_1-CA_3 region of the hippocampus. Such *N*-methyl-D-aspartate effects on [^{3}H]norepinephrine release were significantly higher in the dentate gyrus than in the CA_1-CA_3 area. The *N*-methyl-D-aspartate effects in both hippocampal areas were also reduced significantly by D-2-amino-5-phosphonovaleric acid (50 μM), an antagonist of the *N*-methyl-D-aspartate receptor, and by tetrodotoxin, a blocker of the voltage-dependent Na^+ channels. The extent of this reduction was the same in the dentate gyrus and the CA_1-CA_3 area. Further experiments, conducted in the presence of Mg^{2+}, demonstrated that *N*-methyl-D-aspartic acid increased K^+-induced release of [^{3}H]norepinephrine from dentate gyrus minislices but not from the CA_1-CA_3 area. The results are consistent with the existence of a higher density and/or different subtypes of *N*-methyl-D-aspartate receptors modulating [^{3}H]norepinephrine release in the dentate gyrus as compared with the CA_1-CA_3 hippocampal area.

Several studies have shown that the hippocampus, nucleus of the limbic system, plays an important role in brain functions like memory and learning and in neurological disorders such as epilepsy [1–6]. The excitatory amino acids and noradrenergic neurotransmitter systems present in the hippocampus have been related to these processes.

The hippocampus receives a noradrenergic innervation originating in the locus ceruleus, innervation that is especially dense to the hilus of the dentate gyrus (D.G.)‡ [7]. The hippocampus also has important excitatory amino acid containing neuronal systems [8]. Important fiber tracts, like the perforant path from the entorhinal cortex to the D.G., the mossy fibers, Schaffer collaterals and commissural fibers in the CA_1-CA_3, contain and release glutamate and aspartate. Moreover, the hippocampus presents the highest density of *N*-methyl-D-aspartate (NMDA)-type receptors for excitatory amino acids in the brain [9]. The density of this receptor is not homogeneous, being highest in the CA_1 region [10, 11].

The NMDA-type receptor has been functionally related to the phenomenon of long-term potentiation (LTP) observed in some of the principal excitatory pathways of the hippocampus, located mainly in the CA_1 and D.G. regions [12, 13]. It has also been shown that norepinephrine (NE) modulates LTP responses only in the D.G. [14, 15]. In addition, it has been demonstrated that NMDA can induce the release of [^{3}H]NE previously taken up from whole hippocampal slices [16, 17]. The purpose of the present work was to further characterize this last action of NMDA upon the noradrenergic system, studying whether regional differences exist between the D.G. and CA_1-CA_3 region of the hippocampus which may correlate with the different LTP responses reported in these areas of the hippocampus in the presence of NE [14, 15, 18]. Preliminary results from this study have been reported elsewhere [19, §].

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‡ Abbreviations: D.G., dentate gyrus; NMDA, *N*-methyl-D-aspartate; LTP, long-term potentiation; NE, norepinephrine; APV, D-2-amino-5-phosphonovalerate; KRH, Krebs–Ringer–HEPES; and TTX, tetrodotoxin.

§ Gysling K, Andrés ME and Bustos G, Regulation of ^{3}H-norepinephrine release by *N*-methyl-D-aspartate receptors in slices from dentate gyrus and CA_1-CA_3 area of the rat hippocampus. Abstract, 20th Annual Meeting Soc. Neuroscience, St. Louis, MO, U.S.A., R-229.4, 1990.

MATERIALS AND METHODS

Materials. [^{3}H]NE (43.7 Ci/mmol) was purchased from Dupont (Boston, MA, U.S.A.). D-2-Amino-5-phosphonovalerate (APV), NMDA, and salts for Krebs–Ringer–HEPES (KRH) were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Preparation and incubation of rat hippocampal "minislices". Male Sprague–Dawley rats (250–300 g) were decapitated, the brain was quickly removed and both hippocampi were dissected out at 4°.

Transverse slices (400 μM) were cut with a Sorvall tissue chopper. D.G. and CA_1-CA_3 areas were separated from each slice as described by Burke and Nadler [20].

Twenty minislices of D.G. or ten from the CA_1-CA_3 area were incubated for 30 min at 37° in 2 mL of KRH (composition in mM: 138 NaCl, 5 KCl, 1 $MgSO_4$, 1.2 $CaCl_2$, 1.2 NaH_2PO_4, 20 HEPES and 10 glucose) with 1 mM ascorbic acid at pH 7.4, saturated with 100% O_2 and containing [^{3}H]NE (sp. act. 43.7 Ci/mmol, 2.3×10^{-8} M). After the incubation period, the minislices were transferred rapidly to a lucite chamber with a nylon mesh bottom and washed with 10 mL KRH, before starting the superfusion procedures.

[^{3}H]NE release from D.G. and CA_1-CA_3 minislices. Minislices of both areas were superfused in parallel chambers, with KRH solution continuously oxygenated and prewarmed to 37°, at a constant flow rate of 1 mL/min, maintained by a peristaltic pump. Slices were superfused for 60 min to establish a steady basal release before stimulation. Three samples of 1 min each were collected to determine the spontaneous outflow of [^{3}H]NE. After that, the effects of NMDA (10–50 μM) on spontaneous or induced [^{3}H]NE release were studied.

After each experiment the slices were recovered and the radioactivity remaining in the tissue was extracted with 0.2 M perchloric acid and quantified by liquid scintillation counting.

Calculation of release data. Release of [^{3}H]NE in each minute was calculated and expressed as a percentage of the total radioactivity present in the minislices during that period. To determine stimulated release, the mean of basal efflux of radioactivity during 3 min before the stimulation period was subtracted from each subsequent fraction during and after the period of stimulation. Results are expressed as means ± SEM. A non-parametric Mann–Whitney U test was used for comparison of mean values.

RESULTS

Effect of NMDA upon [^{3}H]NE release from D.G. and CA_1-CA_3 areas. Superfusion of D.G. and CA_1-CA_3 minislices for 5 min with 10–200 μM NMDA in the absence of Mg^{2+} significantly increased [^{3}H]NE efflux (Fig. 1). The NMDA effect was concentration dependent in the two hippocampal areas studied. However, NMDA induced significantly more [^{3}H]-NE release from D.G. than from CA_1-CA_3. This differential effect of NMDA upon NE release in both areas was more manifest when the time–course of [^{3}H]NE release from D.G. and CA_1-CA_3 was analyzed (Fig. 2). The NMDA effect rapidly reached a maximum 1 min after adding NMDA to the superfusing medium in both areas studied. The response to 25 and 50 μM NMDA was about 100% higher in the D.G. than in the CA_1-CA_3 region during the first 2 min of exposure to the drug. Thereafter, the response in the D.G. decreased with time, in spite of the fact that NMDA was still present (Fig. 2). In contrast, the response to NMDA in the CA_1-CA_3 preparation presented a decay only with a high concentration of NMDA (200 μM).

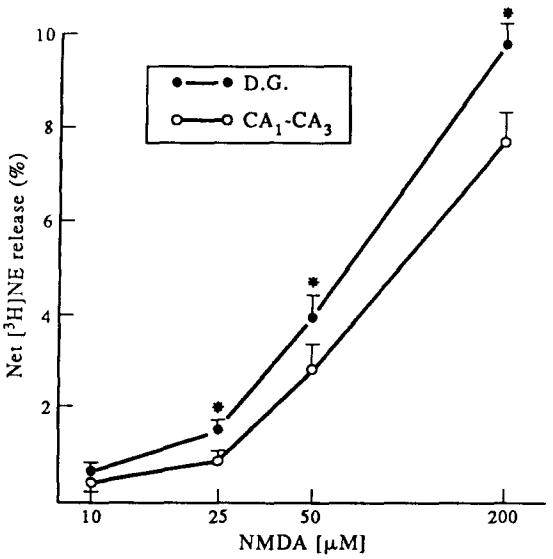

Fig. 1. Concentration–response curve for the effect of NMDA upon the release of [^{3}H]NE from D.G. and CA_1-CA_3 areas of the hippocampus. [^{3}H]NE release was induced by a 5-min exposure to NMDA. Values are the means ± SEM of 3–5 independent experiments. D.G. slices took up an average of 506,000 dpm of [^{3}H]NE, CA_1-CA_3 slices took up an average of 698,000 dpm, and the spontaneous release was 1,813 ± 113 and 1,417 ± 93 dpm/min, respectively. Key: (*) $P < 0.05$ compared with the CA_1-CA_3 value.

The effect of NMDA upon [^{3}H]NE release was observed only in the absence of Mg^{2+}. No significant [^{3}H]NE release induced by 50 μM NMDA was observed in the presence of a normal physiological concentration of Mg^{2+} (1.0 mM), in both areas under study.

Characteristics of the [^{3}H]NE release induced by NMDA in D.G. and CA_1-CA_3 areas. Figure 3 shows that APV, a competitive antagonist of the NMDA receptor, significantly reduced the effect of NMDA on [^{3}H]NE release by 54% in the D.G. and 64% in the CA_1-CA_3 region.

To test whether the NMDA-induced release of [^{3}H]NE from D.G. and CA_1-CA_3 was mediated through the activation of voltage-dependent Na^+ channels, the effect of the specific channel blocker tetrodotoxin (TTX) was studied. As shown in Fig. 3, 0.1 μM TTX significantly reduced by 66 and 58% the [^{3}H]NE release induced by 50 μM NMDA in the D.G. and CA_1-CA_3 area, respectively.

Finally, the omission of Ca^{2+} from the Mg^{2+}-free superfusion medium reduced the NMDA (50 μM) evoked release of [^{3}H]NE by 80.6 ± 14.3% in the D.G. and by 76.8 ± 13.6% in the CA_1-CA_3 region (N = 3).

Effect of NMDA upon K^+-evoked release of [^{3}H]-NE from D.G. and CA_1-CA_3 areas. Potassium depolarization evoked the release of [^{3}H]NE from D.G. and CA_1-CA_3 minislices as a function of the external K^+ concentration used in the superfusion medium (D.G. area = 0.81 ± 0.10, 3.95 ± 0.82 and 10.20 ± 1.57% of [^{3}H]NE release at 25, 40 and

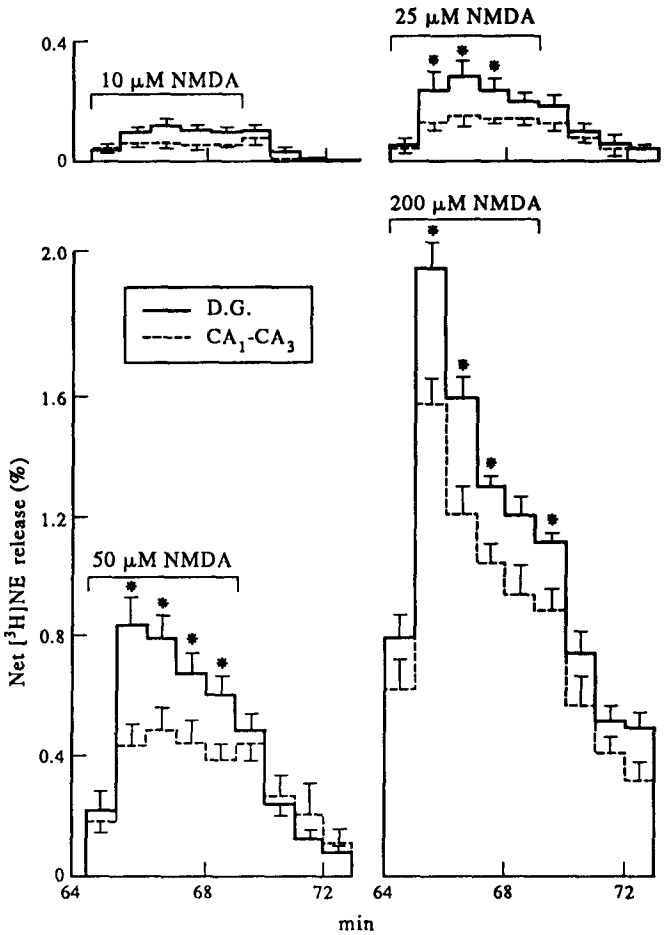

Fig. 2. Time-course of the effect of NMDA upon release of [^{3}H]NE from minislices of the D.G. and CA_1-CA_3 regions of the hippocampus. Minislices obtained as described in Materials and Methods were superfused with KRH and in the absence of Mg^{2+}. In each case, slices were exposed to NMDA (10–200 μM) for a period of 5 min. Basal release before the addition of NMDA was subtracted in each group. Values are the means ± SEM of 3–5 independent experiments. Key: (*) $P < 0.05$ compared with the respective value of the CA_1-CA_3 area.

70 mM K^+, respectively; N = 3) (CA_1-CA_3 = 0.64 ± 0.03, 3.40 ± 0.92 and 11.02 ± 3.37% at 25, 40 and 70 mM K^+, respectively; N = 3). No significant differences were found in [^{3}H]NE release induced by different depolarizing external K^+ concentrations when the D.G. areas were compared with the CA_1-CA_3 area. In other experiments, [^{3}H]NE release induced by 55 mM K^+ was shown to be completely Ca^{2+} dependent in both hippocampal areas (data not shown).

NMDA (50 μM), added before a K^+ (25 mM) stimulus, was found to further increase in the D.G. region the [^{3}H]NE release evoked by this K^+ stimulus (Table 1). In contrast, no significant effect of NMDA upon K^+-evoked release of [^{3}H]NE from CA_1-CA_3 minislices was observed (Table 1).

DISCUSSION

The results of the present study demonstrate that NMDA, a specific agonist of the NMDA subtype receptor for excitatory amino acids, has a differential stimulatory effect upon [^{3}H]NE release in the D.G. and CA_1-CA_3 areas of the rat hippocampus. The characteristics of the NMDA effect upon [^{3}H]NE release from both areas studied were as reported previously for total hippocampus [16, 17, 21]. That is, [^{3}H]NE release evoked by NMDA was calcium dependent and completely abolished by physiological concentrations of magnesium. The NMDA effect was inhibited by APV in both areas, further supporting the hypothesis that this is a selective effect upon NMDA subtype receptors. Finally, the NMDA-induced [^{3}H]NE release was inhibited by 60% in the presence of 0.1 μM TTX in both areas.

The degree of inhibition of the NMDA-evoked release of [^{3}H]NE by APV and TTX was the same in the D.G. and CA_1-CA_3 areas. However, the stimulatory effect of NMDA upon [^{3}H]NE release from the D.G. slices was significantly higher than

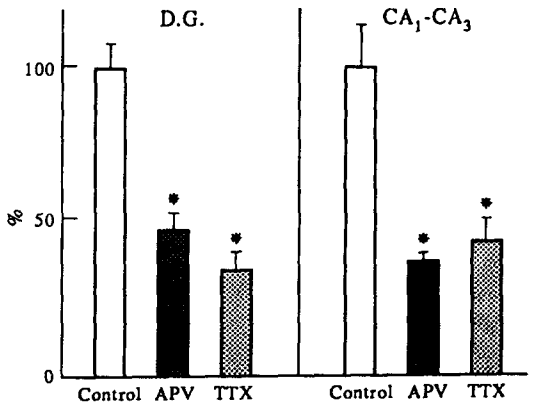

Fig. 3. Effects of 50 μM APV and 0.1 μM TTX upon NMDA-induced release of [^{3}H]NE from D.G. and CA_1-CA_3 regions of the hippocampus. In the control groups of both regions studied, [^{3}H]NE release was induced by the addition of 50 μM NMDA to the superfusion medium for 5 min. In the experimental groups, 50 μM APV or 0.1 μM TTX was added in the superfusion medium 10 min prior to the stimulation with 50 μM NMDA. Values are the means ± SEM of 4–7 independent experiments. One hundred percent corresponds to the [^{3}H]NE release induced by 50 μM NMDA in each area. For the D.G. region, the net [^{3}H]NE release was 14,636 ± 783 dpm and for CA_1-CA_3 9,147 ± 1,120 dpm. Key: (*) $P < 0.01$ compared with the respective control.

Table 1. Effect of NMDA upon [^{3}H]NE release evoked by 25 mM K^+ from the D.G. and CA_1-CA_3 regions of the hippocampus

Hippocampal area	Control S2/S1	Experimental S2/S1
D.G.	0.91 ± 0.07	1.22 ± 0.09*
CA_1-CA_3	0.81 ± 0.06	0.82 ± 0.05

Minislices from each region were superfused with KRH and subjected to two 3-min consecutive stimulation periods with 25 mM K^+ as described in Materials and Methods. The two stimulation periods, S1 and S2, were separated by a 40-min superfusion with normal KRH. In the experimental group, 50 μM NMDA was added 3 min before and during the second stimulus. Values are ratios of K^+-evoked release of net [^{3}H]NE between the second and first stimulation periods (S2/S1). Values are the means ± SEM of four separate and independent experiments.

* $P < 0.05$ when compared with the the control.

that from the CA_1-CA_3 slices, from 25 to 200 μM NMDA. In addition, the time–course of the NMDA effects was also different in the D.G. than in the CA_1-CA_3. The effect of NMDA in the D.G. showed a decay as a function of NMDA concentration. This decay was observed in the CA_1-CA_3 area only at a high NMDA concentration. Finally, NMDA could increase, even in the presence of a physiological concentration of magnesium, the K^+-evoked [^{3}H]-NE release in the D.G. but not in the CA_1-CA_3 area. A higher density of noradrenergic terminals [7] in the D.G. region could account for the observed differential effects. However, this explanation is ruled out by the evidence that depolarization-induced [^{3}H]NE is similar in both areas.

A higher density of NMDA receptors modulating [^{3}H]NE in the D.G. region than in CA_1-CA_3 could explain the stronger effect of NMDA observed in the D.G. area. Indeed, it has been shown that in the hippocampus the NMDA receptors are not uniformly distributed, CA_1 being the region with the highest NMDA receptor density as compared with CA_3 and D.G. [9–11]. Alternatively, it is possible to propose a differential effect of NMDA at the D.G. even with a similar receptor density in D.G. and CA_1-CA_3 areas of the hippocampus. This proposal arises from several findings suggesting the existence of pharmacologically different NMDA receptor subtypes [22–24]. Thus, it has been shown that NMDA-induced release of dopamine and acetylcholine in the striatum was reduced by prior exposure to a low concentration of NMDA and that the addition of glycine reversed the effects of NMDA on dopamine, but not on acetylcholine release [23]. These effects were attributed to the existence of heterogeneity in the NMDA receptors associated to each of these neurotransmitters. In a similar way, different subtypes of NMDA receptors associated to each hippocampal area studied could explain the differential response to NMDA observed in the present work. The recent cloning of the NMDA receptor will permit an investigation of this possibility [25]. In fact, two recent reports provide strong molecular and functional evidence for the existence of distinct subtypes of NMDA receptors with discrete brain distributions [26, 27].

As both D.G. and CA_1-CA_3 minislices include local neuronal circuitry, it is worth considering the possiblity that the activation of post-synaptically located NMDA receptors could cause intrinsic neurons to fire action potentials and trigger the release of diffusible messengers. These diffusible messengers, in turn, could modify the release of NE and differentially act in the D.G. and CA_1-CA_3 areas of the hippocampus. However, if this were the case, it seems unlikely that TTX would similarly affect both hippocampal areas as it did in the present study. Another possibility to consider is that the activation of postsynaptically located NMDA receptors could be coupled to the synthesis and release of neuromodulators such as nitric oxide or arachidonic acid [28, 29]. These neuromodulators could differentially modify the release of NE in D.G. and CA_1-CA_3 hippocampal areas.

Some reports suggest the existence of presynaptic NMDA receptors regulating NE release in cerebral cortical and hippocampal noradrenergic terminals [30, 32]. A higher density or different subtypes of such presynaptic receptors in the D.G. region as compared with the CA_1-CA_3 area could explain the stronger effect of NMDA observed in the D.G. region. This possibility should be investigated further.

In conclusion, the findings reported here are compatible with the idea that NMDA receptors may differentially regulate the release of NE in two

distinct areas of the hippocampus. It is tempting to propose that these responses to NMDA in the D.G. and the CA_1-CA_3 region may be related to the differences in LTP expression usually observed in these two regions of the hippocampus [14, 15, 18].

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REFERENCES

1. Delgado-Escueta AV, Ward A, Woodbury DM and Porter RJ, New wave of research in the epilepsies. In: *Advances in Neurology* (Eds. Delgado-Escueta AV, Ward A, Woodbury DM and Porter RJ), Vol. 44, pp. 3–64. Raven Press, New York, 1986.
2. Rothman SM and Olney JW, Excitotoxicity and the NMDA receptor. *Trends Neurosci* **10**: 299–302, 1987.
3. Deadwyler SA, Hampson RE, Foster TC and Marlow G, The functional significance of long-term potentiation: Relation to sensory processing by hippocampal circuits. In: *Neurology and Neurobiology* (Eds. Landfield PW and Deadwyler SA), Vol. 35, pp. 499–534. A. R. Liss, New York, 1988.
4. Bliss TVP and Lynch MA, Long-term potentiation of synaptic transmission in the hippocampus: Properties and mechanisms. In: *Neurology and Neurobiology* (Eds. Landfield PW and Deadwyler SA), Vol. 35, pp. 3–72. A. R. Liss, New York, 1988.
5. Cotman CW, Monaghan DT and Ganong AH, Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity. *Annu Rev Neurosci* **11**: 61–80, 1988.
6. Cain DP, Long-term potentiation and kindling: How similar are the mechanisms? *Trends Neurosci* **12**: 6–10, 1989.
7. Loy R, Koziell DA, Lindsey JD and Moore RY, Noradrenergic innervation of the adult rat hippocampal formation. *J Comp Neurol* **189**: 699–710, 1980.
8. Ottersen OP and Storm-Mathisen J, Excitatory amino acid pathways in the brain. *Adv Exp Med Biol* **203**: 263–284, 1986.
9. Monaghan DT and Cotman CW, Distribution of *N*-methyl-D-aspartate sensitive L-[^{3}H]glutamate-binding sites in rat brain. *J Neurosci* **5**: 2909–2919, 1985.
10. Maragos WF, Penney JB and Young AB, Anatomic correlation of NMDA and ^{3}H-TCP-labeled receptors in rat brain. *J Neurosci* **8**: 493–501, 1988.
11. Sakurai SY, Cha J-HT, Penney JB and Young AB, Regional distribution and properties of [^{3}H]MK-801 binding sites determined by quantitative autoradiography in rat brain. *Neuroscience* **40**: 533–543, 1991.
12. Dolphin AC, Errington ML and Bliss TVP, Long-term potentiation of the perforant path *in vivo* is associated with increased glutamate release. *Nature* **297**: 496–498, 1982.
13. Bliss TVP, Errington ML and Lynch MA, Calcium-induced long-term potentiation in the dentate gyrus is accompanied by a sustained increase in glutamate release. In: *Excitatory Amino Acid Transmitters* (Eds. Hicks T and Lodge D), pp. 337–340. A. R. Liss, New York, 1986.
14. Bliss TVP, Goddard GV and Riives M, Reduction of long term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. *J Physiol (Lond)* **334**: 474–491, 1983.
15. Stanton PK and Sarvey JM, Depletion of norepinephrine, but not serotonin, reduces long-term potentiation in the dentate gyrus of rat hippocampal slices. *J Neurosci* **5**: 2169–2176, 1985.
16. Vezzani A, Wu H-Q and Samanin R, [^{3}H]Norepinephrine release from hippocampal slices is an *in vitro* biochemical tool for investigating the pharmacological properties of excitatory amino acid receptors. *J Neurochem* **49**: 1438–1442, 1987.
17. Jones SM, Snell LD and Johnson KM, Phencyclidine selectively inhibits *N*-methyl-D-aspartate-induced hippocampal [^{3}H]norepinephrine release. *J Pharmacol Exp Ther* **240**: 492–497, 1986.
18. Johnston D, Hopkins WF and Gray R, Noradrenergic enhancement of long-term synaptic potentiation. In: *Neurology and Neurobiology* (Eds. Landfield PW and Deadwyler SA), Vol. 35, pp. 355–376. A. R. Liss, New York, 1988.
19. Andrés ME and Gysling K, Modulation of ^{3}H-noradrenaline release by *N*-methyl-D-aspartate in slices for CA_1-CA_3 and dentate gyrus of the rat hippocampus. *Arch Biol Med Exp (Santiago)* **22**: R421, 1989.
20. Burke SP and Nadler JV, Regulation of glutamate and aspartate release from slices of the hippocampal CA_1 area: Effects of adenosine and baclofen. *J Neurochem* **51**: 1541–1551, 1988.
21. Schmidt CJ and Taylor VL, Release of [^{3}H]-norepinephrine from rat hippocampal slices by *N*-methyl-D-aspartate: Comparison of the inhibitory effects of Mg^{2+} and MK-801. *Eur J Pharmacol* **156**: 111–120, 1988.
22. Monaghan DT, Olverman HJ, Nguyen L, Watkins JC and Cotman CW, Two classes of *N*-methyl-D-aspartate recognition sites: Differential distribution and differential regulation by glycine. *Proc Natl Acad Sci USA* **85**: 9836–9840, 1988.
23. Cai N-s, Kiss B and Erdö SL, Heterogeneity of *N*-methyl-D-aspartate receptors regulating the release of dopamine and acetylcholine from striatal slices. *J Neurochem* **57**: 2148–2151, 1991.
24. Gonzales RA, Biochemical responses mediated by *N*-methyl-D-aspartate receptors in rat cortical slices are differentially sensitive to magnesium. *J Neurochem* **58**: 579–586, 1992.
25. Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N and Nakanishi S, Molecular cloning and characterization of the rat NMDA receptor. *Nature* **354**: 31–37, 1991.
26. Neguro H, Mori H, Araki K, Kushiya E, Kutsuwada T, Yamazaki M, Kumanishi T, Arakawa M, Sakimkura K and Mishina M, Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature* **357**: 70–74, 1992.
27. Monyer H, Sprengel R, Schoefer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B and Seeburg PH, Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science* **256**: 1217–1221, 1992.
28. Dumuis A, Sebben M, Haynes L, Pin, J-P and Bockaert J, NMDA receptors activate the arachidonic acid cascade system in striatal neurons. *Nature* **336**: 68–70, 1988.
29. Garthwaite J, Charles SL and Chess-Williams R, Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* **336**: 385–388, 1988.
30. Pittaluga A and Raiteri M, Release-enhancing glycine-dependent presynaptic NMDA receptors exist on noradrenergic terminals of hippocampus. *Eur J Pharmacol* **191**: 231–324, 1990.
31. Fink K, Bönisch H and Göthert M, Presynaptic NMDA receptors stimulate noradrenaline release in the cerebral cortex. *Eur J Pharmacol* **185**: 115–117, 1990.
32. Wang JKT, Andrews H and Thukral V, Presynaptic glutamate receptors regulate noradrenaline release from isolated nerve terminals. *J Neurochem* **58**: 204–211, 1992.