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Involvement of intracellular Ca^{2+} stores in 3, 4-diaminopyridine-evoked [^{3}H]norepinephrine release[1]

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KEY WORDS norepinephrine; hippocampus; 3, 4-diaminopyridine; reserpine; BAPTA; dantrolene; desipramine

AIM: To study the mechanism for 3, 4-diaminopyridine (DAP) evoking external Ca^{2+}-independent release of [^{3}H] norepinephrine ([^{3}H] NE). **METHODS:** Rat hippocampal slices were preincubated with [^{3}H] NE and superfused with medium. [^{3}H] NE release was determined. **RESULTS:** Under Ca^{2+}-free conditions, DAP evoked [^{3}H] NE release. In rats pretreated by reserpine, the effect of DAP was no longer detectable. Ca^{2+} chelator BAPTA-AM potently inhibited DAP-evoked [^{3}H] NE release. Desipramine 100 - 500 μmol · L^{-1} strongly induced [^{3}H] NE release in a concentration-dependent manner, whereas caffeine 30 - 70 mmol · L^{-1} was slightly effective on [^{3}H]NE release. The blocker of Ca^{2+}-induced Ca^{2+} releasable stores, dantrolene sodium did not attenuate DAP-evoked [^{3}H]NE release. **CONCLUSION:** In the absence of extracellular Ca^{2+}, DAP evokes exocytotic release of [^{3}H]NE from synaptic vesicles through liberation of internal Ca^{2+} from inositol 1, 4, 5-trisphosphate-sensitive Ca^{2+} stores.

Our previous study found that 3, 4-diaminopyridine (DAP) exhibited particularly potent facilitatory effects on the spontaneous ^{3}H outflow from rabbit hippocampal slices preincubated with [^{3}H] norepinephrine ([^{3}H] NE)[1]. This effect was tetrodotoxin (TTX) sensitive and modulated by presynaptic α_2-adrenoceptors, κ-opioid and adenosine A_1 receptors; It was strongly enhanced after activation of protein kinase C (PKC) with phorbol ester, or reduced by various PKC inhibitors. These observations indicate the involvement of action potential-evoked exocytotic release of [^{3}H] NE following DAP application. In the absence of extracellular Ca^{2+}, DAP also induced [^{3}H] NE release from rabbit and rat hippocampal slices preincubated with [^{3}H] NE[2] and phorbol ester enhanced this evoked [^{3}H] NE release[3]. The aim of this study was to study the mechanism of DAP-induced external Ca^{2+}-independent [^{3}H]NE release from rat hippocampal slices.

MATERIALS AND METHODS

Chemicals 1-(7, 8-^{3}H)norepinephrine ([^{3}H]NE, Amersham, UK); DAP, desipramine, caffeine, dantrolene sodium (Sigma, USA); Tetraacetoxy methyl ester of 1, 2-bis(2-aminophenoxy) ethane-*N*, *N*, *N'*, *N'*-tetraacetic acid (BAPTA-AM, Molecular Probes Inc, USA). Stock solutions of the drugs were prepared in water except BAPTA-AM dissolved in Me_2SO.

Procedure Hippocampal slices (0.35 mm thick), prepared from Sprague-Dawley rats (♂, certificate number 02-35-2, n = 25), were preincubated with [^{3}H] NE 0.1 μmol · L^{-1} and then superfused with medium 0.7 mL · min^{-1}. Samples were collected every 5 min. After 60 min of superfusion the slices were exposed to drugs to induce transmitter release. At the end of superfusion, the slices were solubilized in 0.5 mL Soluene-350 (Packard). The ^{3}H content of the solubilized slices and the superfusion samples was determined with a liquid scintillation counter. The preincubation medium: NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, disodium edetate 0.03 mmol · L^{-1} (saturated with 5 % CO_2 in O_2; pH 7.4). In Ca^{2+}-free superfusion medium, $CaCl_2$ was replaced by egtazic acid 1 mmol · L^{-1}. In the experiment with BAPTA-AM, the slices were preincubated with BAPTA-AM 0.4 mmol · L^{-1} for 2 h (controls with 0.5 % Me_2SO) before incubation with [^{3}H]NE. Desipramine 1 μmol · L^{-1} was present throughout the superfusion to block reuptake system of NE[2].

Calculation The fractional rate of ^{3}H outflow was calculated as ^{3}H outflow per 5 min divided by the ^{3}H content in the slices at the start of the respective 5 min period. The drug-evoked ^{3}H ourflow was estimated by subtracting the basal outflow from the total outflow of the 50

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min after the onset of the drug. The basal outflow of ^3H was assumed to decline linearly from the fraction 55 – 60 min, to the fraction 110 – 115 min of superfusion. The evoked outflow of ^3H was expressed as % of the ^3H content of the slices at the onset of the drug. Results were shown as $\bar{x} \pm s$. The significance of differences between the groups were determined by t test[4].

RESULTS

***Effects of reserpine and BAPTA-AM on DAP-evoked* [^{3}H] NE release in the absence of extracellular Ca^{2+}** To test whether DAP-evoked external Ca^{2+}-independent release of [^{3}H] NE is exocytosis from synaptic vesicles, the rats were injected sc with reserpine 10 mg·kg^{-1} 12 h before death. When NE stored in synaptic vesicles was depleted by reserpine, DAP-evoked [^{3}H]NE release was no longer detectable (Tab 1).

Tab 1. Effects of reserpine and BAPTA-AM on DAP-evoked [^{3}H]NE release in the absence of extracellular Ca^{2+}. Hippocampal slices were preincubated with [^{3}H] NE and superfused continuously. After 60 min of superfusion the slices were exposed to DAP to induce [^{3}H]NE release. $n = 4 - 6$ slices from 2 rats, $\bar{x} \pm s$. $^cP < 0.01$.

Drug/ μmol·L^{-1}	Evoked outflow of ^{3}H/ % of tissue ^{3}H
DAP 300	9.6 ± 0.4
+ reserpine	0.13 ± 0.10^c
DAP 200	4.10 ± 0.22
+ BAPTA-AM	0.41 ± 0.05^c

The effects of Ca^{2+} chelator BAPTA-AM were studied to examine the necessity of elevation of cytosol free Ca^{2+} for the induction of DAP-evoked [^{3}H] NE release. BAPTA-AM 0 4 mmol · L^{-1} inhibited the DAP-evoked release of [^{3}H] NE by 90 % of controls (Tab 1).

Effects of desipramine and caffeine on [^{3}H] NE release in the absence of extracellular Ca^{2+} At 60 min of superfusion, addition of desipramine 100 μmol·L^{-1} for 10 min to the Ca^{2+}-free superfusion medium (with egtazic acid 1 mmol·L^{-1}) enhanced ^{3}H outflow over basal values. The outflow reached the maximum at 65 – 70 min. Subsequently the ^{3}H outflow slowly returned to basal values within 50 min after the addition of desipramine (Fig 1).

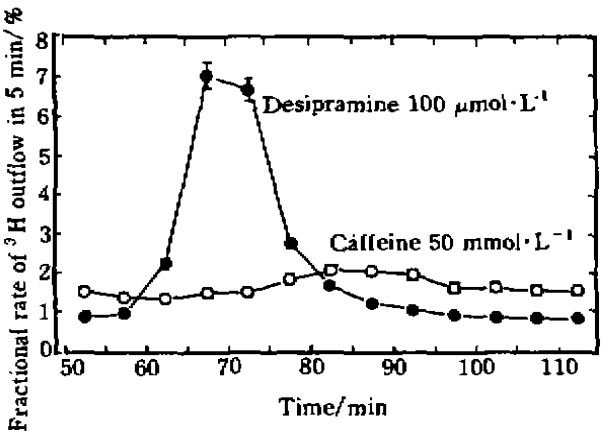

Fig 1. Desipramine- and caffeine-induced release of [^{3}H]NE from rat hippocampal slices preincubated with [^{3}H]NE and superfused continuously. After 60 min of superfusion the slices were exposed to desipramine 100 μmol·L^{-1} or caffeine 50 mmol·L^{-1} in Ca^{2+}-free medium containing egtazic acid 1 mmol·L^{-1}. $n = 4$ slices from 2 rats, $\bar{x} \pm s$.

Addition of caffeine 50 mmol·L^{-1} to the Ca^{2+}-free medium containing egtazic acid 1 mmol·L^{-1} at 60 min of superfusion very slowly and slightly increased ^{3}H outflow, which reached the maximum at 85 – 90 min and gradually returned to basal level after the drug was withdrawn (Fig 1).

Both the enhancements of ^{3}H outflow by desipramine and caffeine were concentration-dependent and increased linearly from 100 to 500 μmol · L^{-1}, and from 30 to 70 mmol · L^{-1} respectively (Tab 2).

Tab 2. Desipramine- and caffeine-induced [^{3}H]NE release from rat hippocampal slices. After 60 min of superfusion the slices were exposed to various concentrations of desipramine or caffeine. $n = 4 - 10$ slices from 3 rats, $\bar{x} \pm s$.

Drug	Evoked outflow of ^{3}H/ % of tissue ^{3}H
Desipramine/μmol·L^{-1}	
100	22.6 ± 1.0
200	40.2 ± 5.1
300	52.3 ± 2.3
400	61.4 ± 5.0
500	74.4 ± 1.3
Caffeine/mmol·L^{-1}	
30	0.45 ± 0.20
50	2.44 ± 0.21
70	6.0 ± 1.0

Effects of dantrolene sodium on DAP-evoked external Ca^{2+}-independent release of [^{3}H]NE We used dantrolene Na^+ (saturated in medium) to block Ca^{2+}-induced Ca^{2+} release. In the absence of extracellular of Ca^{2+}, DAP-evoked ^{3}H outflow was unchanged (4.8 ± 0.4 % in dantrolene Na^+ group and 4.6 ± 0.4 % in control group).

DISCUSSION

We have previously shown[1] that [^{3}H] NE release was evoked by DAP from rabbit hippocampal slices preincubated with [^{3}H] NE. We proposed that in the presence of extracellular Ca^{2+}, DAP by blocking K^+ currents, depolarizes the neuronal membrane, induces Ca^{2+} influx and exocytosis of NE storage vesicles.

In the present investigation, it was observed that in the absence of extracellular Ca^{2+}, DAP significantly evoked [^{3}H] NE release, but became ineffective when NE stored in synaptic vesicles was depleted by reserpine pretreated[5]. This indicates that under Ca^{2+}-free conditions, DAP-evoked [^{3}H] NE release was also a vesicular component. Any possible involvement of extracellular Ca^{2+} in the process of DAP-evoked [^{3}H] NE release can be excluded by egtazic acid 1 mmol·L^{-1} contained in medium[6]. The question arises, by which mechanisms DAP evoked exocytotic release of [^{3}H]NE, whether it is dependent from liberation of intracellular Ca^{2+} stores? This speculation is supported by the experiment with BAPTA-AM.

BAPTA-AM is hydrolyzed after crossing the membrane and dissociates Ca^{2+} chelator BAPTA. The Ca^{2+} liberated from intracellular stores was rapidly buffered by BAPTA, so that no appreciable accumulation of Ca^{2+} occurred during repetitive stimulation[7]. As shown in Tab 1, the release of [^{3}H]NE induced by DAP was significantly inhibited in the slices pretreated with BAPTA-AM, indicating an involvement of liberation of intracellular Ca^{2+} stores in the process of DAP-evoked release of [^{3}H]NE.

In the present study, using high concentration desipramine[8] to stimulate the inositol 1, 4, 5-trisphosphate (IP_3)-sensitive Ca^{2+} stores[9], we observed a strong external Ca^{2+}-independent release of [^{3}H] NE from the slices, whereas the effect of caffeine[10] by stimulating Ca^{2+}-induced Ca^{2+} releasable stores[11] on inducing [^{3}H]NE release was very slight even in very high concentration of the drug. This suggests that in the absence of external Ca^{2+}, the elevation of cytosol free Ca^{2+}, which is necessary for stimulation-evoked [^{3}H] NE release from adrenergic terminals of hippocampal slices, is mostly from the IP_3-sensitive Ca^{2+} stores. It has been reported that dantrolene sodium blocked Ca^{2+}-induced Ca^{2+} release from endoplasmic reticulum in neurons[12]. In the present investigation, dantrolene Na^+ did not show any inhibitory effect on DAP-evoked release of [^{3}H]NE, suggesting that in the absence of extracellular Ca^{2+}, Ca^{2+}-induced Ca^{2+} release might not be involved in the mechanism of DAP-evoked [^{3}H]NE release. Conclusion, in the absence of extracellular Ca^{2+}, DAP by liberation of intracellular Ca^{2+} from IP_3-sensitive Ca^{2+} stores evokes exocytotic release of [^{3}H] NE from synaptic vesicles.

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胞内储备钙参与 3,4-二氨基吡啶诱发去甲肾上腺素释放[1]

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关键词 去甲肾上腺素; 海马; 3,4-二氨基吡啶; 利血平; BAPTA; 丹曲林; 地昔帕明

目的: 用大鼠海马脑片研究 3,4-二氨基吡啶(DAP)诱发去甲肾上腺素胞外钙-不依赖释放的机制 方法: 大鼠海马脑片用[^{3}H]NE 孵育后, 进行表面灌流, 测[^{3}H]NE 释放. 结果: 在胞外无钙条件下, DAP 能显著加强[^{3}H]NE 释放, 当用利血平使囊泡[^{3}H]NE 排空, 则 DAP 作用消失, 用高浓度地昔帕明刺激 IP_3-敏感的胞内 Ca^{2+} 储备库, 能有力地增强[^{3}H]NE 释放, 而高浓度咖啡因对[^{3}H]NE 释放只有很微弱的作用, 丹曲林钠对 DAP 诱发[^{3}H]NE 释放无任何抑制作用. 结论: 在胞外无钙条件下, DAP 通过 IP_3-敏感的 Ca^{2+} 储备库释放 Ca^{2+}, 从而诱发囊泡内的去甲肾上腺素释放.

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Effects of methylflavonolamine on free intracellular calcium in isolated embryonic rat brain cells

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KEY WORDS methylflavonolamine; Fura-2; calcium; flunarizine; glutamate; brain; cultured cells

AIM: To observe the effects of methylflavonolamine (MFA) on free intracellular calcium concentration ($[Ca^{2+}]_i$) of isolated embryonic rat brain cells in presence and absence of high extracellular potassium and *L*-glutamate. **METHODS**: $[Ca^{2+}]_i$ was measured in a spectrofluorophotometer by preloading the cells with calcium sensitive fluorescent indicator Fura 2-AM. **RESULTS**: Resting $[Ca^{2+}]_i$ was 197 ± 20 nmol $\cdot L^{-1}$ ($n = 44$) in the presence of Ca^{2+} 1.3 mmol $\cdot L^{-1}$ in Hanks' solution. MFA 0.15 mmol $\cdot L^{-1}$ had no effect on the resting $[Ca^{2+}]_i$. When extracellular Ca^{2+} was 1.3 mmol $\cdot L^{-1}$, MFA (0.03 - 0.3 mmol $\cdot L^{-1}$) concentration-dependently inhibited the $[Ca^{2+}]_i$ elevation induced by high extracellular potassium, with an IC_{50} value of 0.14 (95 % confidence limits: 0.05 - 0.42) mmol $\cdot L^{-1}$. At higher concentration (0.15 - 0.30 mmol $\cdot L^{-1}$), MFA decreased *L*-glutamate-induced $[Ca^{2+}]_i$ elevation, with an IC_{50} of 0.20 (95 % confidence limits: 0.01 - 3.40) mmol $\cdot L^{-1}$. **CONCLUSION**: MFA inhibited Ca^{2+} influx through voltage-dependent calcium channel and, at higher concentration, through receptor-operated calcium channel in the embryonic rat brain cells.

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