اثر نترودوتوکسین و کادمیم روی آزاد شدن و متابولیسم سیستم‌های سروتونرژیک و نورآدنرژیک هیپوکامپ

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چکیده :

با استفاده از تکنیک میکرو دیالیز مغز، ارتباط بین تحریک نورونی و آزادشدن و متابولیسم نوروترانمیترهای هیپوکامپ مورد مطالعه قرار گرفت. تترودوتوکسین *(TT)* بطور داخل هسته‌ای داده شد و یون کادمیم (Ca^{++}) با یون کلسیم *(Ca++)* در *CSF* مصنوعی میکرودیالیز معاوضه گردید. اثرات این دو دارو روی سیستم‌های سروتوژنیک و نورآدنرژیک هیپوکامپ مطالعه شد.

موش سفید (۲۵۰ تا ۳۰۰ گرم) انتخاب و پراب‌های میکرودیالیز در هیپوکامپ قدامی در شرایط بیهوشی (کلیال هیدارت) کاسته شد. بعد از دوران رکاوری ۵- هیدروکسی تریپتامین *(5-HT)* ۵- هیدارکسی اندول استیک اسید *(5-HIAA)* نورآدرنالین *(NA)* و دی هیدراکسی فنیل اسید استیک *(DOPAC)* با استفاده از *HPLC* با آشکارساز الکتروشیمیایی اندازه‌گیری شد. از مسدودکننده‌های برداشت *HT* 5- *(Citalopram)* و *NA(Dssipramine)* نیز استفاده شد.

بعد از ثبوت خط پایه و برداشت سه نمونه شاهد *TTX* داده شد و این سبب کاهش خط پایه *5-HT* به *5-HIAA* ٪۲۹ $\downarrow$ ۴($p<$ ۰/۰۵) به *NA* ٪۸۷ $\downarrow$ ۷($p<$ ۰/۰۵)، ٪۲۴ $\downarrow$ ۳($p<$ ۰/۰۵) و *DOPAC* به ٪۵۵ $\downarrow$ ۱۷($p<$ ۰/۰۵) در عرض ۱۰۰ دقیقه گردید (n=۶) زمانی که Cd^{++} جایگزین Ca^{++} گردید کاهش خط پایه *5-HT* به ٪۳۸ $\downarrow$ ۸($p<$ ۰/۰۵) در عرض ۱۰۰ دقیقه گردید. (n=۶) .

نتایج نشان می‌دهد که آزاد شدن *5-HT* و *NA* در هیپوکامپ روندی فعال است و بستگی به دشارژ شدن پایانه عصبی این سیستم‌ها و ورود یون Ca^{++} به این پایانه‌ها دارد. متابولیسم این سیستم‌ها متعاقب به آزاد شدن، در هرمورد کاهش می‌یابد. با این حال نتایج نیاز مبرم یون Ca^{++} برای فعال‌تر شدن آنزیم‌های متابولیکی این سیستم‌ها را توصیه می‌کند.

کلید واژه‌ها: ۱ـ میکرو دیالیز ۲ـ تترودوکسین ۳ـ کادمیوم ۴ـ نورآدرنرژیک هیپوکامپی ۵ـ سیستم‌های سروتونرژیک

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THE EFFECT OF TETRODOTOXIN AND CADMIUM ON HIPPOCAMPAL NORADERNALINE AND 5-HYDROXYTRYPTAMINE RELEASE AND TURNOVER.

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ABSTRACT

Using the technique of in vivo microdialysis, we examined the relationship between neuronal firing and neurotransmitter release and metabolism in the hippocampus, The neurotoxin tetrodotoxin (TTX) was applied locally to block voltage-sensitive sodium channels, and extracellular Ca++ was replaced with cadmium (Cd++) to inhibit Ca++ dependant neurotransmitter release. The effectes of these drugs on both noradrenergic and serotonergic transmitter system were evaluated. The results show that 5-HT and NA release in hippocampal nerve terminal are active process and depend on the cell firing and influx of Ca++ ions prior to release. 5-HTand NA synthesis and turnover are also decreased by TTX and Cd++, these decays are greater in the absence of Ca++ions. this suggest an essential role of ca++ ions for hippocampal monaoamine synthesis and turnover.

Key Words: 1) Microdialysis 2) tetrodotoxin 3) Cadmium 4) Hippocampal noradrenergic 5) serotonergic systems.

INTRODUCTION

The classical monoamines neurotransmitters 5-HT and NA are probably the most studied of all CNS transmitter substances. In the central nervous system both NA and 5-HT have been implicated in a wide range of behavioural and psychological functions such as aggressive and predatory behaviour , anxiety and depression. In spite of such

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diverse functions attributed to 5-HT and NA, obtaining direct evidence has been hampered by the difficulty inherent in the in vivo estimation of these neurotransmitters. Electrophysiological recording from single units of both these systems in freely moving animals [1, 32] has been utilised to evaluate the activity of these systems; thistechnique is unable to detect changes in the amount of transmitter release, synthesis, turnover resulting from changes in synaptic terminals. Various neurochemical approaches, such as prsh-pull perfusion[12,30] and in vivo voltammetry [3,22] have also been used to monitor the release of brain neurotransmitters. However, these techniques either produce tissue damage in the implanted area of brain [13,25,30] because of the continuous tissue wash and the size of push-pull cannulae, or are unable to monitor extracellular NA and 5-HT levels directly, partly because of the similarity of the oxidation potential of the metabolites and the neurotransmitter [3,4,19,21,31]. Recently, the development of in vivo microdialysis techniques for direct measurement of the extracellular concentrations of the neurotransmitters and their metabolites have presented promising tools for investigating the conditions under which the activity of these neurotransmitter systems is altered as well as how this activity is regulated. the use of the dialysis technique also provided several advantages over other in vivo techniques. The most important are its high resolution and the ability to measure several different analytes in the same sample in freely moving animal so that behaviour can be correlated with chemical changes in the brain. Instead of such advantages, brain dialysis is aninvasive in nature. It attempts to monitor the release of transmitter from never terminals by inserting into the brain a probe several orders of magnitude larger than the biological structure than the biological structure under study. Thus due to this nature it is necessary to establish specific criteria for evaluation of neurotransmitter output as estimated by dialysis. These include the use of the selective voltage dependent Na+ channel blocker tetrodotoxin (TTX), replacement of cd++ with ca++ in perfusing medium for assessment of the physiological release process and activity of the synthetic enzyme[9,16,29,33,34]. The results obtained from these brain dialysis studies depends on at least three variables: type of probe, post implantation interval, and whether anaesthetised or freely moving animals are used.

MATERIAL & METHOD

Rats (250-300g) were implanted with microdialysis probes in the ventral hippocampus under chloral hydrate anaesthesia (500 mg/kg i.p.), and allowed to recover overnight. Samples of dialysate

were collected every 20 minutes from the unanaesthetised and freely moving animals and assayed for 5-HT, 5-HIAA, NA and DOPAC using HPLC with electrochemical detection. Two separate HPLC assay systems were used one of which measured 5-HT and 5-HIAA, and the other measured NA and DOPAC. Since basal levels of the transmitters were close to the limit of detection of the assay system, citalopram (μ1 M), or desipramine (10 M) was added in most experiments to the perfusion medium for the assay of 5-HT and NA respectively; these drugs are selective uptake blockers for these transmitters. The neuronal origin of the compounds was verified eitheer by replacement of Ca++ with Cd++ in the perfusing medium or by the use of the Na++ channel blocker TTX (1 μM). The mean of the three basal samples was calculated and the value of all individual samples was expressed as a percentage of this mean. For statistical significance of the effect of a drug comparisons were made with the last sample before the addition of the drug using absolute values and the paired student's test.

RESULTS

Serotonergic nerve terminals

In ordet to determine to what extent the basal levels of 5-HT and 5-HIAA in hippocampal dialysate are impulse traffic-dependent the voltage-dependent Na+ channel blocker TTX was infused. Localinfusion of TTX (1 M) through the dialysis probe caused an initial rapid decrease in the concentration of 5-HT which was followed by a further slower reduction to 24±5% ($p<0.006$, n=3) by 80 min, the duration of experiment. The initial rapid reduction of 5-HT to 33±6% occurred in the first 20 min sample.

Local administration of TTX caused a gradual and much smaller reduction in the concentration of 5-HIAA to 84±3% ($p<0.001$, n=8) by 80 min (Fig.1.).

In order to determine the Ca++ -dependence of basal 5-HT and 5-HIAA, Ca++ was replaced with Cd++ in the Ringer solution. This caused and initial rapid decrease to 53±18% in the first 20 min sample in the concentration of 5-HT, was followed by a slower reduction to 23±8% ($p<0.01$, n=3) by 100 min; 5-HIAA was reduced to 18±5% ($p<0.003$, n=6) by 100 min (Fig.2.).

Noradrenergic nerve terminals

Local infusion of TTX (1 μM) through the dialysis probe caused an initial rapid decrease to 49±5% within the first 20 min sample in the basal concentration of NA followed by a further slow reduction to 24±3% ($p<-.02$, n=3) by 100 min. The concentration of DOPAC was reduced to 55±17% ($p<0.001$, n=8) by 100 min (Fig.3.).

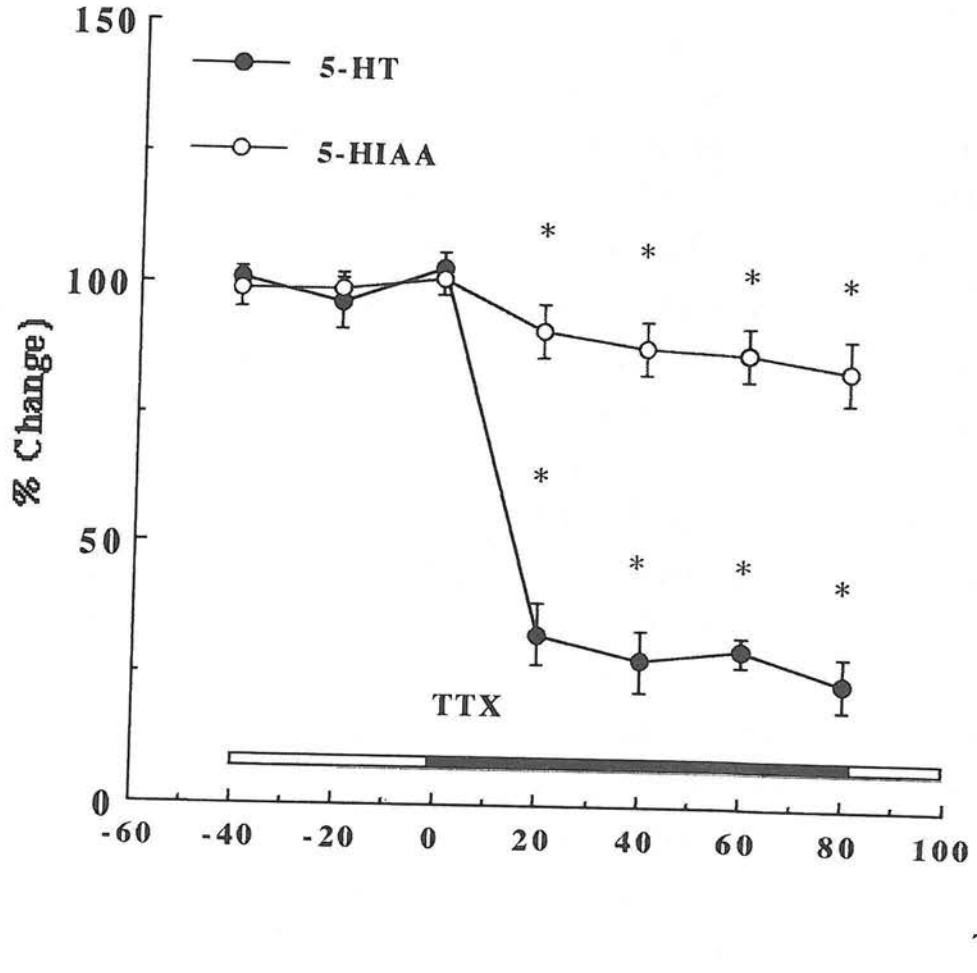

Figure 1. The effect of TTX on the basal level of 5-HT and 5-HIAA. Changes in the concentration of 5-HT are shown as a percentage of the last sample taken before the infusion. The solid bar shows the infusion of TTX. $p<0.005$ ($n=8$ for 5-HIAA and 3 for 5-HT) compared to the last sample taken before TTX.*

Replacement of Ca^{++} with Cd^{++} in theperfusion medium caused an initial rapid decrease the basal concentration of NA followed by a progressive slowreduction to 32±8% ($p<0.01$, n=3) by 100 min. There was a more gradual reduction in DOPAC level to 47±5% ($p<0.0003$, n=6) by 100 min (Fig.4).

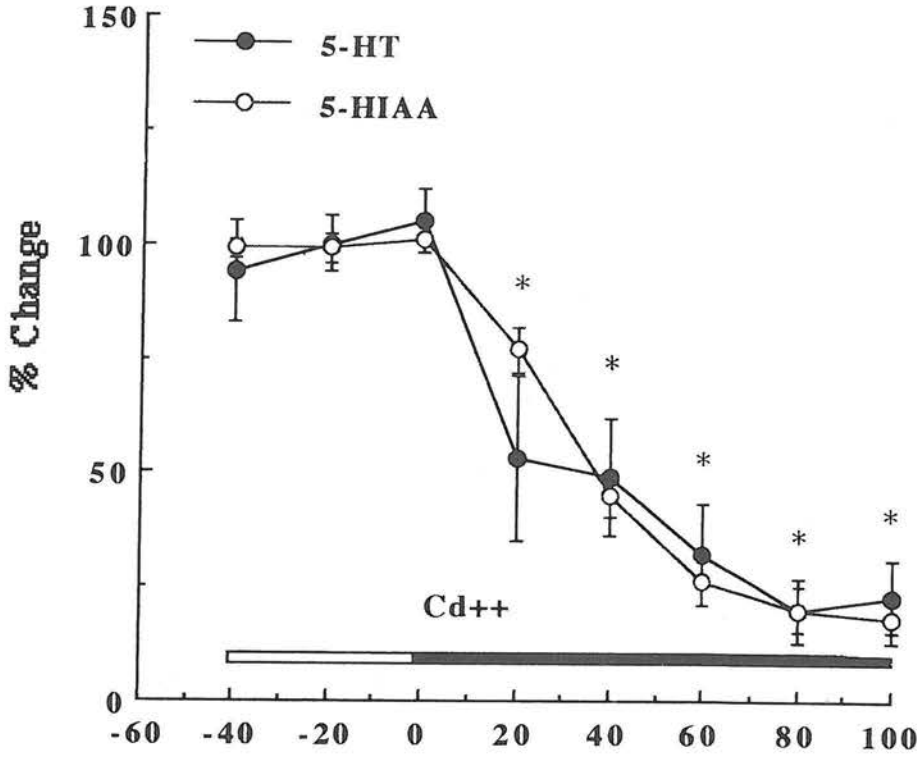

*Figure 2. The effect of the replacement of Ca++ with Cd++ in the perfusion medium on the basal level of 5-HT and d5-HIAA. The solid bar shows the infusion of Cd++. *p<0.05(n=6 for 5HIAA and 3 for 5-HT) compared to the last sample taken before Cd++.*

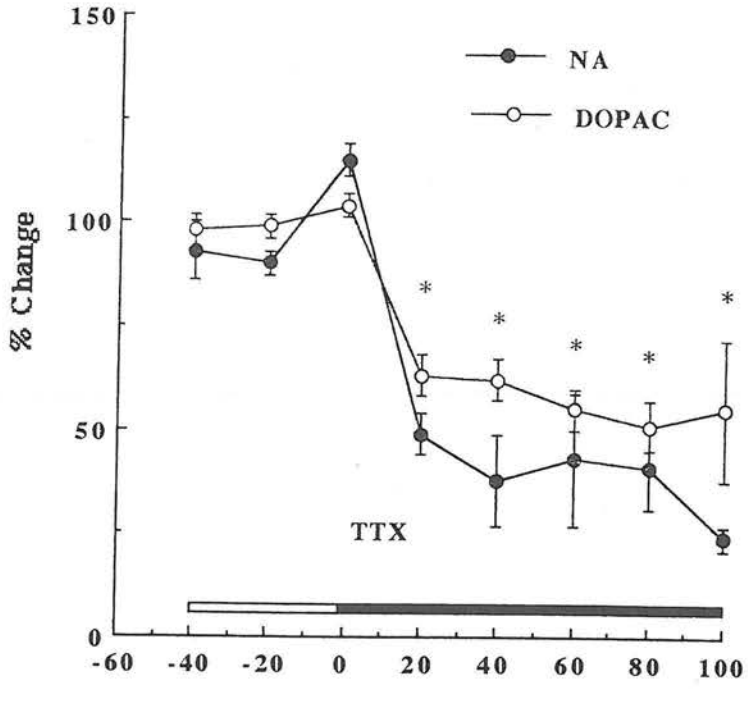

*Figure 3.The effect of TTX on the basal level of NA and DOPAC. The solid bar shows the infusion of TTX. *p<0.05 (n=8 for DOPAC and 3 for NA) compard to the last sample taken befor TTX.*

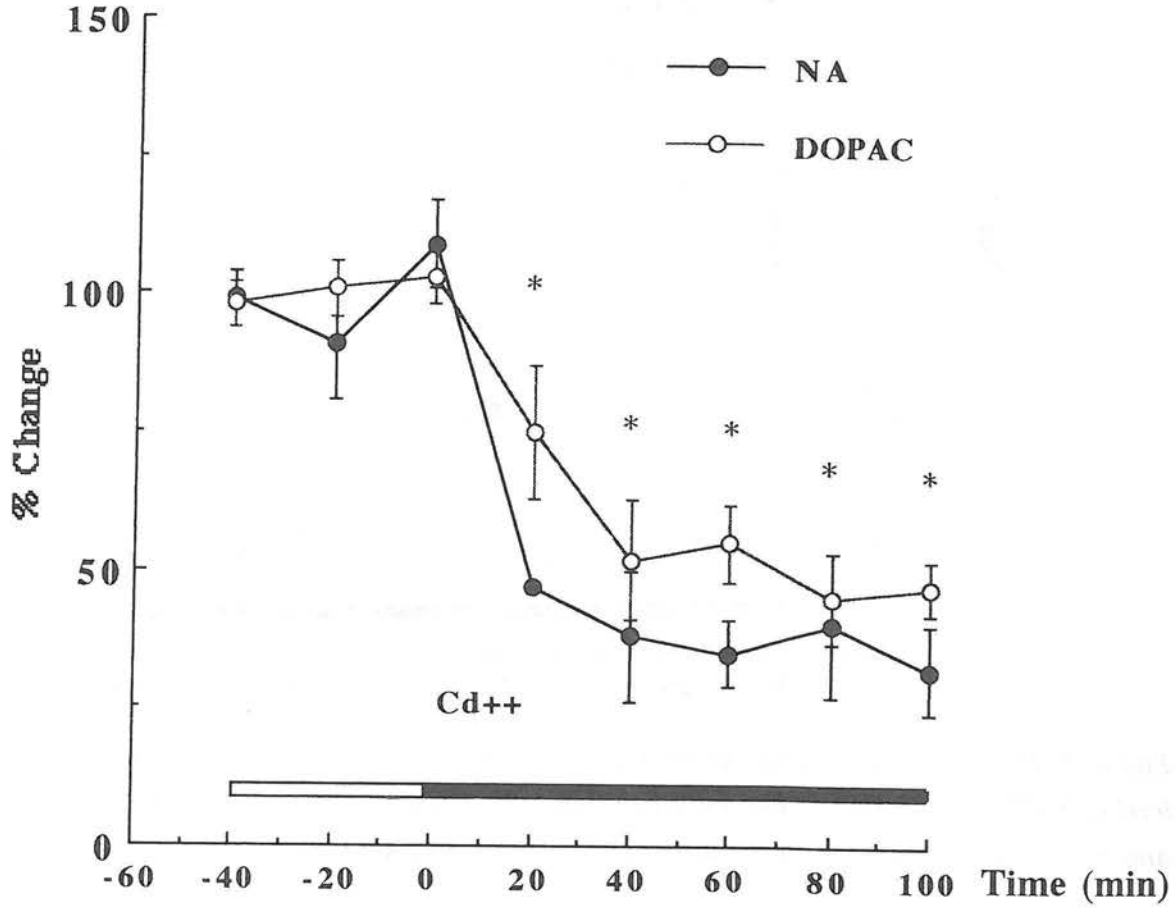

*Figure 4.The effect of the replacement of Ca++ with Cd++ in the perfusion medium on the basal level of NA and DOPAC. The solid bar shows the infusion of Cd++. *p<0.05 (n=6 for DOPAC and 3 for NA) compared to the last sample taken before Cd.++*

DISCUSSION

In the present study in vivo microdialysis has been used to monitor the basal levels of hippocampal 5HT and NA their metabolites in unanaesthetised freely moving rats. The voltage-dependent Na^+ channel blocker, TTX, was used locally to determine to what extent the basal levels of 5-HT and NA are impulse-dependent. For the metabolites TTX was used to determine the link between impulse traffic, transmitter synthesis and turnover. Previous studies have shown that TTX reduces the spontaneous release in vivo of acetylcholine (Ach) [6,7], DA [34], NA [33] and 5-HT [2,9,17,29], as well as the electrically evoked release of several amino acids, including GABA [15]. The reduction to 24%

of foth 5-HT and NA by TTX confirms the dependence of their release on nerve impulse traffic as also found by [161]others[2,17,29]. The remaining 5-HT and NA is probably due to the fact that TTX did not reach all the nerve terminals.

The smaller reduction of the two metabolites levels suggests that synthesis is less closely coupled to impulse traffic than release. There is however a considerable difference between metabolites, 5-HIAA showing a much smaller reduction after TTX than DOPAC. This suggests that in the NA pathway there is close coupling between impulse traffic, neurotransmitter release and synthesis. The evedence concerning the relation between impulse traffic and 5-HT turnover is less clear. TTX (1 μM) had no effect on 5-HIAA levels in hippocampus in anaesthetised rats [29] or caudate-putamen of unanaesthetised rats [17].

Release of transmitter from the nerve terminals on arrival of an action potential depends on the influx of calcium [18]. The requirement for calcium has been shown using microdialysis for the release of Ach[5], DA [14], NA [20,33], and 5-HT [2,17]. The incomplete abolition of 5-HT and NA release by replacement of calcium with cadmium is again could be due to the fact that not all nerve terminals were reached by the cadmium.

As with TTX, the effect of calcium replacement with cadmium has different effects on transmitters and metabolites. In the case of DOPAC the reduction is slightly less than that of NA. In noradrergic neurones calcium in addition to triggering exocytotic NA release, stimulates a pre synaptic adenylate cyclase and is required for activation of tyrosine hydroxylase[26] the rate-limiting enzyme for NA synthesis.

By contrast 5-HIAA, which showed little reduction with TTX, is reduced to a greater extent than is 5-HT by cadmium. This suggests an important role for intracellular calcium in 5-HT synthesis. Other work has shown that electrical stimulation of the dorsal raphe increases the level of 5-HT by a mechanism that is attenuated by calcium free perfusate[28,29], although after replacement of Ca^{++} with Na^{++} ions there is no change in 5-HIAA in caudate putamen[17]. Tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis, requires caleium, and an increase in synthesis has an absolute requirement for extracellular calcium ions[10,11]. Thus not only release but also synthesis of both neurotransmitters in hippocampal nerve terminals are calcium dependent.

5-HIAA is the main deaminated metabolite of 5-HT via monoamine oxidase pathway, and represents the turnover of both newly synthesised 5-HT that has not been released and 5-HT which has been taken up by the nerve terminals after release. In some studies 5-HIAA levels have been

used as an index of the activity of serotonergic neurones [3,24].

Tissue levels of 5-HIAA represent overall 5-HT turnover[27]; in the presence of a 5-HT re-uptake blocker the re-entry of released 5-HT is prevented and the 5-HIAA levels reflect only the turnover of the newly synthesised 5-HT and not released 5-HT. 5-HIAA levels, in the presence of uptake blocker may thus serve as an index of 5-HT synthesis. A more direct measurement of the activity of TrH, is the 8accumulation of 5-HTP in the presence of m-hydroxybenzyl-hydrazine, the inhibitor for the decarboxy lase enzyme [8]. The effects of TTX and cadmium on 5-HIAA in the presence of citalopram suggest that there is basal rate of synthesis, which is largely independent of impulse traffic, but is very sensitive to the extracellular concentration of Ca^{++}.

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