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Effects of Single and Repeated Electroconvulsive Shock on the Acetylcholine and Polyamine Contents in Temporal Cortex and Decorticated Cerebrum of Mice

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ABSTRACT

There are some rather conflicting reports correlating ECS-induced changes of brain acetylcholine, and recently, Zawia and Bondy(1990) proposed the biological role of polyamine system in the long-term adaptive responses of brain to electrical stimulation. This study was undertaken to evaluate the effects of a single or repeated ECS(10mA, 100cps, 1sec; 5 ECS spread out over 9 days) on the brain acetylcholine(ACh) and polyamine contents of male mice.

The ACh contents of temporal cortex(TCx) and decorticated cerebrum(dc-CB) were markedly increased by 79.9% and 49.4%, respectively, 10 and 30min after ECS, and the increases were significantly attenuated with repeated 5 ECS, particularly in dc-CB. The putrescine concentrations of both TCx and dc-CB were little different and not affected by 1 ECS or 5 ECS.

But the spermidine(Sd) concentration was higher in dc-CB and spermine(Sm) higher in TCx. While they were moderately decreased after 1 ECS, and their decreases were accentuated after 5 ECS, particularly in dc-CB. Sm(30mg/kg, i.p. inject. 30min before ECS) did not affect the ECS-induced increase of ACh content.

Thease results suggest that both of brain ACh and polyamine may be implicated with the long-term adaptive responses to electrical stimulation

Key Words: Electroconvulsive shock, Acetylcholine, Polyamine

INTRODUCTION

The last few years have seen a marked increase in the research on the biochemical and pharmacological consequences of repeated electroconvulsive shock(ECS)(Small *et al.*, 1986; Green and Nutt, 1987). Electroconvulsive therapy(ECT) has consistently proved to be as effective or more effective than antidepressants medications(Brandson *et al.*, 1984) and produces short-term benefit in a significant proportion of acute schizophrenia(Small, 1985).

But ECT is often regarded as the most controversial treatment in psychiatry, and the controversy is fuelled with the criticism that we are ignorant of how ECT works, both in producing therapeutic change and with respect to adverse effects(Sackeim, 1988).

Since Echlin(1959) suggested an epileptogenic potential of acetylcholine(ACh) in the cerebral cortex, significant experimental contributions by Snead(1983), Girgis(1985) and Wasterlain *et al.* (1986) placed ACh into a conceptual framework underlying the epileptogenesis. Turski *et al.* (1989) demonstrated that the piloca-

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rpine-induced seizure model may be of value in designing new therapeutic approaches to epilepsy.

However, there are some rather conflicting reports correlating brain ACh with seizure susceptibility in convulsive mice strains(Karczmar 1974) and ECS-induced changes of brain ACh concentration(Atterwill, 1984).

By the way, the brain contents of polyamines such as spermidine(Sd) and spermine(Sm) were decreased by about 25% after the electrical stimulation of the precentral gyrus in the Rhesus monkey(Russell *et al.*, 1974), while the brain ornithine decar-boxylase(ODC) and S-adenosylmethionine decarboxylase(SAM-DC) activities, the biosynthesis enzymes of polyamine(PA) were markedly enhanced by ECS (Pajunen *et al.*, 1978).

In addition, polyamine, particularly spermine, most effectively inhibited choline uptake by forebrain synaptosomes but slightly inhibited the uptake of dopamine or GABA(Law *et al.*, 1984).

Therefore, this study was undertaken to evaluate the ECS-induced changes of brain acetylcholine and polyamine contents.

MATERIALS AND METHODS

Materials

Male ICR mice, weighing 15-20g, were supplied from Korea Experimental Animal Lab. Company.

Acetylcholine, acetylcholinesterase(purified from E. electricus), choline oxidase, horseradish peroxidase(type II, HRP), and 5-amino-1,2,3,4-tetrahydronaphthalazindione-1,4 (luminol), and the hydrochlorides of putrescine, spermidine and spermine were purchased from Sigma. 1,8-Diaminooctane and 4-fluoro-3-nitrobenzotrifluoride (FNBT) were from Aldrich.

Other chemicals were analytical or high performance liquid chromatography (HPLC) grade.

Treatments of animals

Ten mice were kept in a cage to be acclimated to the light-dark cycle(light, 12 hr from 7 AM to 7 PM; dark, remained 12 hr) for one week before being studied. And mice were given a single ECS(13 mA, 100 cps, 1 sec) through bilateral ear-clip electrodes using a model 7801 ECT unit of Ugo Balsile Comp., or a series of repeated 5 ECS(a single ECS per day with a interval of a day) and then were sacrificed 10, 30, and 60 minutes after the last ECS. The control mice for repeated 5 ECS were given a series of 4 ECS and the shamed final ECS in the same schedule of the repeated 5 ECS treatment, and while mice were handled for the final ECS, the ear-clips were placed but no current passed. And spermine hydrochloride, 30mg/kg, was intraperitoneally injected in 0.85% NaCl saline 30 min before given ECS.

Measurement of brain acetylcholine content

The measurement was based upon the method of Israel and Lesbats(1987) and all procedures were accomplished below 2°C.

Extraction and oxidation: Tissues of 100mg or more were minced in 10 volumes of ice-chilled 5% trichloracetic acid(TCA), standed for 60 min, and spun out at 15,000×g for 5 min. The about 200 ul of TCA supernatant was washed 5 times with 4ml of chilled water-saturated diethy ether for control of its pH up to 4, and the the residual ether was completely evaporated, and 10 ul of 0.5% sodium metaperiodate was added to 100 ul of the remained extract to eliminate the reducing substances which interfere with the chemiluminescent reaction.

Luminescent measurement of ACh: The reaction medium were at first, consisted of 0.4ml of 200mM sodium phosphate buffer, 15 ul of assay chemiluminescent mixture and 10 ul of the extract, and finally added with 100ul of acetylcholinesterase(AChE) reagent. And then the luminescent count were integrated for 60 sec using a Biolumat LB 2500C of Berthold Company. The recovery rate through this experiment was about 72.1±8.4%. The assay luminescent mixture was prepared with 100 ul distilled water(DW) of 25 unit choline oxidase, 50 ul DW of about 10 unit HRP, and 100 ul of 18 ug luminol in 0.2M Tris buffer, pH 8.6, just before an ACh determination. And the AChE reagent were prepared as following: a 250 ul of AChE (1000 unit per ml) in chilled DW was passed through a coarse Sephadex G50 column of 5ml volume equilibrated in DW to get a low blank value, and the column was furhter eluted with 1.45ml DW before collecting the enzyme in 0.

7ml, which was stored below −40°C. Immediately before the assay, the working reagent was prepared by dilution of the stock solution with 19 volumes of 0.2 M sodium phosphate butter, pH 8.6.

Measurement of brain polyamine content

High Performance Liquid Chromatography (HPLC) system: The system was consisted of a Gilson HPLC pump, a Rheodyne 7125 injection valve, a Erma ERC ODS-1161 column(3 um; 6× 100mm), a Knauer Model 87 variable UV/VIS spectrodetector, and a Linear dual-channel chart recorder.

Polyamine extraction and HPLC analysis: The extraction procedure was carried out below 2°C according to the method of Choi *et al.* (1988). Derivatization and HPLC analysis of polyamines were based upon the method originally described by Spragg and Hutchings(1983) and first adopted by Choi *et al.* (1988) to biological samples. The analytic procedures are briefly as following. Mouse brain was extirpated immediately after decapitation, froze in dry ice powder, and dissected for the isolation of both temporal cortex and decerebrated cerebrum, and then the isolated tissues were stored below −25°C for 7 days or less before polyamine assay.

The frozen tissues were thawed in flaked ice and homogenized with a teflon homogenizer in 4 volumes of chilled 0.4M perchloric acid containing 2mM disodium EDTA and diaminooctane of 50-100ug as an internal standard. One ml of the homogenate was spun out at 15,000×g for 10 min, and 100 ul of the supernatant was evaporated by a Speed Vac dryer of Sarvant comp., and the dry residue was dissolved in 100 ul of 1M sodium bicarbonate, and then derivatized with 300 ul of FNBT reagent(a mixture of 10 ul FNBT and one ml of dimethyl sulfoxide) at 60°C for 20 min. At the end of derivatization, 40 ul of 1M histidine in 1M sodium bicarbonate was added to the reaction mixture, and then the derivatization continued for a further 5 min to scavenge the excess FNBT.

After cooling the mixture in a ice basket, the N-2'-nitro-4'-trifluoromethylphenyl derivatives of polyamines were extracted twice with 2ml of 2-methylbutane. After centrifugation at 3,000×g for 10 min, the organic phase was evaporated with streams of nitrogen gas, and the residue was reconstituted with 1ml of HPLC-grade methanol. 20 ul of the methanol solution was applied to a reversed phase HPLC analysis.

The HPLC system was loaded with the methanol sample, and the polyamine derivatives were isocratically eluted with acetonitrile-DW(80: 20, v/v) solvent at flow rate of 1.2ml/min within 20 min, showing the complete resolution. The effluent was monitored by a UV/VIS detector set at 242 nm and a dual-channel chart recorder. The capacity factors of polyamine derivatives range from 4.12 to 19.75, the recovery rates of polyamines were greater than 94.0%, and the detection limit was less than 10 picomole on column with a signal/noise ratio of 5.

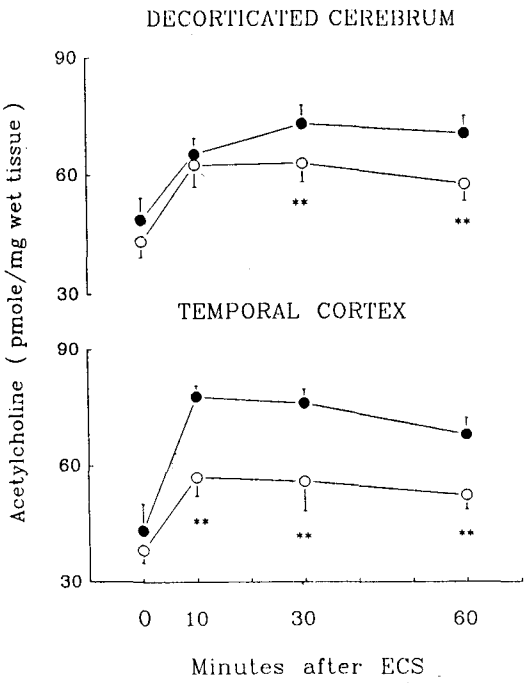

Fig. 1. Influences of single(filled circle) and repeated(open circle) ECS on the acetylcholine content of the decorticated cerebrum and temporal cortex.
Each circle and bar represents the mean and standard error of 6 data.
***, ** and * indicate $p < 0.005$, $p < 0.01$ and $p < 0.1$, respectively.

The amount of each polyamine per g of wet tissue was first estimated directly from the calibration curve based on the peak height, and the value was corrected by the recovery factor of diaminooctane applied as an authentic internal standard.

RESULTS

ECS-induced change of brain acetylcholine content

The ACh contents of temporal cortex(TCx) and decorticated cerebrum(dc-CB) in normal mice were 43.29±5.8 and 48.99±5.4 pmole/wet g, respectively.

The contents of both TCx and dc-CB was significantly increased by 49.4% and 79.9% within 30 min after a single ECS(1 ECS). But the 1 ECS-induced increase of TCx were significantly attenuated by a series of repeated ECS(a single ECS per day with one day interval for 9 days: 5 ECS) and the 1 ECS-induced increase of dc-CB, to a much greater extent, was markedly inhibited after 5 ECS, as hsown in Fig. 1.

ECS-induced changes of brain polyamine contents

The putrescing(PT), spermidine(Sd) and spermine(Sm) contents of dc-CB in normal mice were 21.00±1.3, 290.51±20.6 and 292.03±12.0 nmole/wet g, respectively, and those contents of TCx in normal mice were 19.79±1.4, 190.67±13.3 and 352.99±16.0 nmole/wet g, respectively, like the past report (Russel *et al.*, 1974) showing that Sd concentration was higher in white matter and Sm higher in grey matter (Fig. 2, 3 and 4).

The PT contents of both dc-CB and TCx were little affected by 1 ECS and also by 5 ECS (Fig. 2).

But the contents of both Sd and Sm in dc-CB or TCx were moderately decreased after 1 ECS and the decreasing effect of 1 ECS significantly accentuated by repeated ECS; particularly, to a

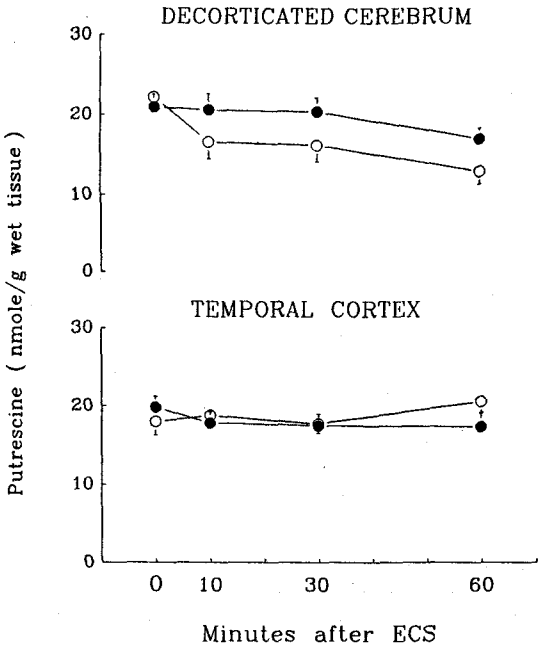

Fig. 2. Influences of single(filled circle) and repeated(open circle) ECS on the putrescine content of the decorticated cerebrum and temporal cortex.

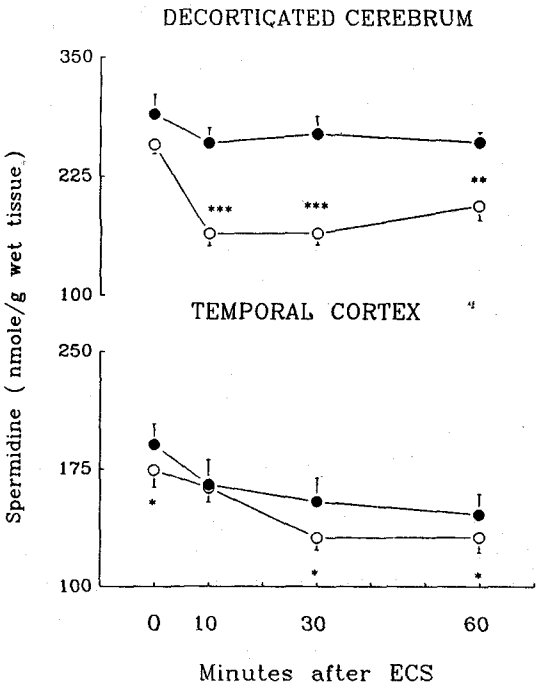

Fig. 3. Influences of single(filled circle) and repeated(open circle) ECS on the spermidine content of the decorticated cerebrum and temporal cortex.

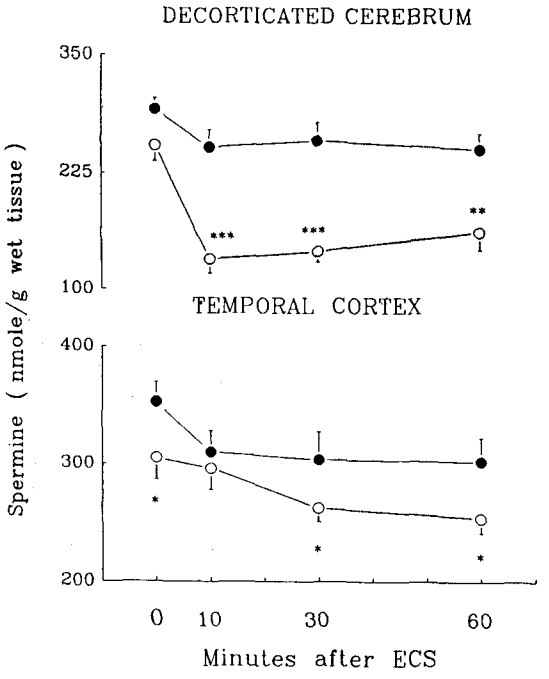

Fig. 4. Influences of single (filled circle) and repeated (open circle) ECS on the spermine content of the decorticated cerebrum and temporal cortex.

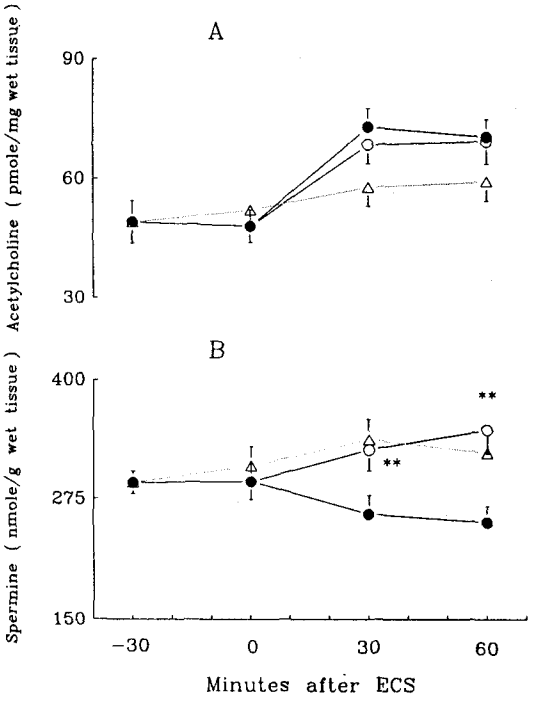

Fig. 5. Influence of spermine on the increase of acetylcholine(A) and the decrease of spermine (B) in the cerebral hemisphere induced by single ECS.
△: Spermine, 30mg/kg IP injection
●: ECS, 30 min after saline injection
○: ECS, 30 min after spermine 30mg/kg IP injection

greater extent in dc-CB(Fig. 3 and 4).

In addition, the Sm contents in both dc-CB and TCx were significantly decreased by 4 ECS in a series of 5 ECS and the contents were not recovered up to the normal values by 24 hrs; that is, the Sm contents of mouse brain just before the last ECS in a series of repeated 5 ECS were significantly lower than those of the normal mice(Fig. 3 and 4) suggesting that the mode of repeated ECS-related change of brain polyamine metabolism might be somewhat different from that of the ECS-induced change of brain ACh, of which the TCx-content was more greatly depressed after 5 ECS compared with the changes in dc-CB(Fig. 1).

Spermine effect on the ECS-induced change of brain acetylcholine content

As shown in Fig. 5, Sm(spermine, 30mg/kg i.p. inject. 30 min before ECS) did not significantly affect the 1 ECS-induced change of ACh content of the whole cerebrum.

DISCUSSION

Although the polyamine synthesis and concentrations are increased in proportion to the growing rate of organs, the polyamine concentrations of nonproliferating brain are among the highest in the body(Russell *at al.*, 1974). And there appears to be little information on the ECS-related changes of brain polyamine metabolism, and if any, most of them are confined to the ODC acitivity(Pajunen *et al.*, 1978; Baudry *et al.*, 1986; Zawia and Bondy, 1990), with an except of an work of Russell *et al.* (1974), showing the regional differences of the brain polyamine contents and the decreases of both Sd and Sm contents of precentral gyrus induced by elec-

trostimulation.

In addition, many studies(Snead, 1983; Girgis, 1985; Wasterlain *et al.*, 1986) placed ACh into a conceptual framework underlying the epileptogenesis, and recently, Turski *et al.* (1989) demonstrated that pilocarpine-induced seizure may be a valuable experimental model in evaluating the action modes of antiepileptic drugs, but there are some rather conflicting reports correlating brain ACh with ECS(Atterwill, 1984).

By the way, the brain contents of polyamines such as spermidine(Sd) and spermine(Sm) were decreasedby about 25% after the electrical stimulation of the precentral gyrus in the Rhesus monkey(Russell *et al.*, 1974), while the brain ornithine decarboxylase(ODC) and S-adenosylmethionine decarboxylase(SAM-DC) activities, the biosynthesis enzymes of polyamine(PA) were markedly enhanced by ECS(Pajunen *et al.*, 1978).

While Green *et al.* (1987) and Green and Vincent(1987) demonstrated that repeated ECS spread over 10 days markedly inhibited K^+-evoked releases of γ-aminobutyric acid(GABA), 5-hydroxytryptamine(5-HT), and norepinephrine(NE), respectively, from cortical slices of rat brains. And Iqbal and Koenig(1985) demonstrated that enhanced polyamine synthesis was required for the increases of K^+-stimulated $^{45}Ca^{2+}$ influx and efflux and the Ca^{2+}-dependent release of GABA and NE in the synptosome obtained from rat cerebral cortex.

In addition, polyamine, particularly spermine, most effectively inhibited choline uptake by forebrain synaptosomes but slightly inhibited the uptake of dopamine or GABA(Law *et al.*, 1984).

The present study was carried out to evaluate the short-term changes of the brain acetylcholine and polyamine contents of male mice induced by repeated ECS.

As shown in Fig. 1, a single ECS(1-ECS) rapidly increased the ACh contents of both temporal cortex and decorticated cerebrum by 79.9 % and 49.4%, respectively, but the ECS-induced increase was significantly attenuated after a series of 5 ECS(5-ECS) given spread out over 10 days with one day interval. This result seems to be linked with the past few reports showing that the acetylcholinesterase activity of rat cortex was increased after a single ECS and yet unchanged after repeated ECS(Longoni *et al.*, 1976) and that the acetylcholinesterase activity of midbrain and hippocampus showed a sustained decrease following a single ECS(Appleyard *et al.*, 1986).

Although most reports citated by Atterwill (1984) in a review suggested a reduction in the rodent brain ACh content during ECS or shortly thereafter, there were some conflicting reports of the ECS-related change of brain ACh content (Appleyard *et al.*, 1986) and as a matter of fact, little valuable evidences for changes in presynaptic cholinergic mechanisms following repeated ECS(Lerer, 1987). Anyway, the present results seem to suggest a part of the neuronal adaptation in response to repeated ECS. Furthermore, the overall relationship of anticonvulsant potencies for Na^+-dependent high affinity choline uptake in mouse hippocampal synaptosomes followed the same order as their anticonvulsive potencies(Miller and Richter, 1985), and polyamines, particularly spermine, effectively inhibited the synaptosomal uptake of choline and to a lesser extent, GABA and NE (Law *et al.*, 1984). But Porta *et al.* (1983) suggested that methionine sulfoximine-induced seizure was ascribed to the increases of cerebral Sd and Sm levels.

In this study, the brain content of putrescin, a diamine, was little affected by 1 ECS or 5 ECS, but following 1 ECS, the contents of both Sd and Sm, polyamines, showed similarly their moderated decrease, and the ECS-induced moderate decreases of them were markedly accentuated in the cortex of temporal lobe and to a greater extent, in the decorticated cerebrum after the last ECS in a series of 5 ECS. And even if the 1 ECS-induced decrease of cerebral Sm content was reversed by exogenous Sm of excess amount(30 mg/kg), the extent of the 1 ECS-induced increase of cerebral ACh content was not changed by exogenous Sm. In considerstion of the inhibitory effect of polyamines on synaptosomal choline-uptake(Law *et al.*, 1984), the present results suggest that the great attenuation of the 1 ECS-induced increase of the brain ACh content after 5 ECS may be independent upon the modulation by polyamines of neuronal choline uptake, enforcing the investigation on the influence of altered polyamine metabolism in the brain on the seizure threshold and

acetylcholine metabolism.

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= 국문초록 =

경련성 전기충격에 의하여 나타나는 측뇌-피질과 피질을 제외한 대뇌의 Acetylcholine 및 Polyamine 함량-변동에 관한 연구

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뇌에 대한 경련성 전기충격(electroconvulsive shock: ECS)을 이용한 치료가 시작된 후, 50여년에 걸쳐 이의 생물학적 작용에 대한 연구가 있었으나, 이렇다할 결과가 아직 없으며, 특히 뇌의 신경전달물질중 가장 중요한 것으로 인정되는 acetylcholine의 함량이 ECS로 증가되는지 감소되는지 확실치 않다. 더욱이, 대체로 조직의 재생능에 비례하는 함량의 증감을 보이는 polyamine함량이 가장 재생능이 미약한 뇌에 고농도로 있으며 뇌의 국소에 따라서도 그 함량에 큰 차가 있고, 뇌의 polyamine-합성 또한 ECS에 의하여 촉진된다고 하는데, 최근에 Zawia와 Bondy는 polyamine-대사가 뇌-신경의 장기적 적응현상에 관련됨을 제시하였다. 따라서 본 연구에서는 웅성 ICR계 생쥐에 ECS(13 mA, 100 cps, 1 sec)를 단회(1 ECS)-부하하여 나타나는 변동을 검색하고 그 결과를 5회(매일 1회씩 이틀마다 5회: 5 ECS)-부하하여 얻은 것과 비교-검토하였다.

측뇌-피질(temporal cortex: TCx)과 피질을 제거한 대뇌(decorticated cerebrum: dc-CB)의 acetylcholine(ACh)함량이 1 ECS 부하후 각각 10분 및 30분에 79.9 및 49.4% 증가되었으며, 이 증가가 5 ECS 부하시에는 유의하게 감약되었던 바, 특히 TCx에서 더욱 현저하였다. Polyamine의 경우, putrescine함량은 TCx 및 dc-CB에서 1 ECS 및 5 ECS 어느 부하에 의하여도 별 변동을 보이지 않았으나 spermidine(Sd) 및 spermine(Sm)은 1 ECS 후에 다소 감소되었을 뿐 아니라 그 감소의 크기가 5 ECS 후에는 현저히 증폭되었고, 특히 dc-CB에서 더욱 현저하였다. 또한 ECS를 4회-부하하고 24시간 후의 Sd 및 Sm 함량은 ACh 함량과 달리 정상치에 비하여 유의하게 낮았으며, 따라서 ECS에 의한 ACh함량-변동에 미치는 Sm(30 mg/kg, 복강내주사)의 영향을 관찰하였던 바 별 변화를 볼 수 없었다.

이상의 성적은 반복되는 ECS에 대하여 대뇌의 ACh 및 polyamine대사가 각각 특이적인 적응성 변동을 보임을 시사하는 것으로 사료된다.