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Effects of CDP-Choline, Aminoguanidine and Difluoromethylornithine on the ECS-induced Impairment of Active Conditioned Response Retention

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ABSTRACT

The training of male wistar rats for active conditioned response (ACR) was performed by one daily training session of 30 consecutive trials for 10 successive days using a two-way shuttle box, and the rats that showed 10 or more ACRs on the last day were treated for further 10 days with electroconvulsive shock (ECS : 50 mA, 0.5 msec; 100 Hz; 1.5 sec) and the following compounds. On the 20th day, all the rats were tested for the ACR rention.

The ECS regimens were one ECS per day for 10 days with one day interval (5×ECS), one ECS at 3 hrs (ECS-3h), and one ECS at 24 hrs (ECS-24h), respectively, before the ACR retention test.

And CDP-choline (cc: 250 mg/kg), spermine (SM: 10 mg/kg), α-difluoromethylornithine (DO: 250 mg/kg), or aminoguanidine (AG: 100 mg/kg) was administered by one daily i.p. injection for 10 days.

The ACR number (13.7±1.0) obtained on the last training day was increased by 37.23% on the 20th day in the control rats. And the ACR increase was significantly suppressed by 5-ECS, ECS-3h, CC, or SM but was little affected by ECS-24h, DO, or AG.

However, the 5-ECS induced impairment of ACR retention was significantly suppressed by AG, SM, and CC in the order of potency but was little affected by DFMO. And the ECS-3h induced impairment was moderately worsened by SM or AG.

The acetylcholine (ACh) of the rat hypothalamus (HT), hippocampus (HC), and entorhinal cortex (EC) was markedly increased by CC and moderately increased by SM, but little affected by ECS-3h, ECS-24h, DO, or AG. But 5×ECS slightly increased the ACh content. The brain putrescine (Pt) content was significantly increased by AG and little affected by CC, SM, or DO. But the 5×ECS markedly decreased the brain Pt content, and the decrease was significantly suppressed by CC, SM, or AG.

CC induced the marked increases of the spermidine (Sd) and spermine (Sm) contents of all the areas. SM increased the Sd contents of all the areas and the EC-Sm content. DO decreased the brain Sd and Sm contents. And AG increased the HT-Sd content and the Sm contents of all the brain areas.

The 5×ECS induced decrease of the HC-Sm content was suppressed by CC, SM and AG.

These results suggest that the improving effect of aminoguanidine on the 5×ECS induced impairment of ACR retention may be ascribed in part to its activity as a diamine oxidase inhibitor.

Key Words: Active conditioned response, Electroconvulsive shock, Acetylcholine, CDP-choline, Polyamine, Aminoguanidine

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INTRODUCTION

Recent estimates suggest that 12 percent of the population of the United States or 24 million people are over the age of 65 (Cummings and Benson, 1983) and by the 2030, 17-20 per cent of the population or 51 million people will be at least 65 years of age (Wells, 1981). The scourage of old age is customarily characterized by ever deepening amnesia (Smith, 1989).

However, electroconvulsive shock (ECS) therapy that has the superiority to other treatment approaches on severe endogenous depression (Brandson *et al.*, 1984) and produces short-term benefit in a significant proportion of acute schizophrenia (Small, 1985), has the misfortune to precipitate the disturbance of cognitive functions (Squire, 1977; 1984).

In spite of a number of researches on the biochemical and pharmacological consequences of ECS (Small *et al.*, 1986; Green and Nutt, 1987), ECS therapy is often regarded as the most controversial treatment in psychiatry, and the controversy is fuelled with the criticism that we are ignorant of how ECS works, both in producing therapeutic change and with respect to adverse effects (Sackeim, 1988).

By the way, although not all investigators would agree with the conclusion that, "if there is a key transmitter for memory... the best bet for that neurotransmitter would be acetylcholine (ACh)" (Davies, 1985; Bartus *et al.*, 1985), interest in cholinergic systems and memory has surged dramatically following reporting that Alzheimer's disease is accompanied by a decline in cholinergic function (Davies and Maloney, 1976; Perry *et al.*, 1977). Further, extensive evidences also indicate that the functioning of cholinergic system declines with aging (Gibson *et al.*, 1987; Decker, 1987). On the other hand, polyamines (PA) also tend to decrease during aging (Nitta *et al.*, 1979), inhibit the choline uptake by rat brain synaptosomes (Law *et al.*, 1984), induce seizures (Porta *et al.*, 1983), and activate the N-methyl-D-aspartate (NMDA) receptor (Reynolds, 1990).

Therefore, this study was undertaken to evaluate the ECS-induced impairment of active conditioned response (ACR) in reference with the changes of brain ACh and PA levels in male Wistar rats.

MATERIALS AND METHODS

Materials

Male Wistar rats, weighing 200-250 g, were supplied from Korea Experimental Animal Lab. Company. Two rats were kept in a cage under a light-dark cycle with light on from 07: 00 to 19: 00 h and received food and water *ad libitum* for one week before being studied.

Acetylcholine, acetylcholinesterase (purified from E. electricus), choline oxidase, horseradish peroxidase (type II, HRP), 5-amino-2,3-dihydro-1, 4-phthalazinedione (luminol), aminoguanidine, and the hydrochlorides of putrescine, spermidine and spermine were purchased from Sigma.

1,8-Diaminooctane and 4-fluore-3-nitrobenzotrifluoride (FNBT) were from Aldrich. And DL-*α*-difluoromethylornithine was generously provided by Merrel Dow Laboratories (Cincinnati, OH, USA). Other chemicals were analytical or high performace liquid chromatography (HPLC) grade.

Training of active conditioned response and retention test of the response

The training and retention test of active conditioned response (ACR) were carried using a Lafayette two-way shuttle box (model 85103) divided into two 30×20×20 cm compartments with a connecting opening.

After 5 min adaptation in the box, light conditional stimulus (CS) and unconditioned stimulus (UCS), a 0.8 mA electric foot-shock through the grid floor, were sequentially presented for a maximum of 5 sec or until the animal escaped into the opposite compartment, respectively.

The inter-trial interval was 15 sec. Each animal was trained for 10 consecutive days with 30 trials in a daily training session. Retention test was carried out on the 10th day after the last training session. The schedule of the ACR training and retention test was as shown in Fig. 1.

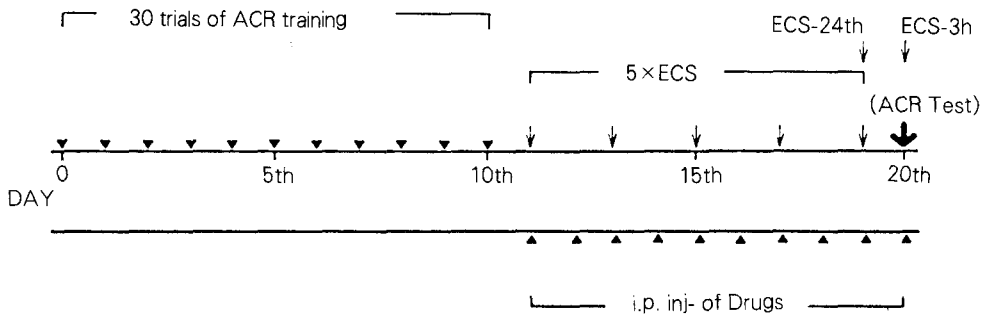
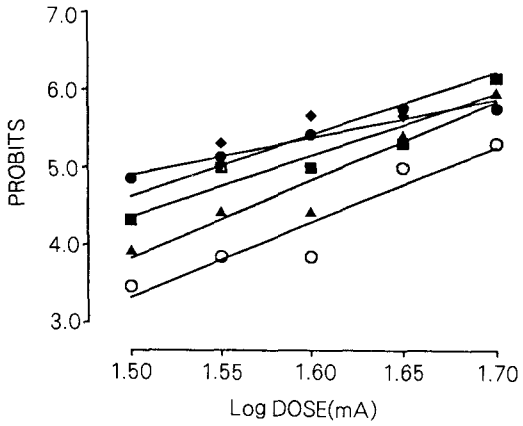


Fig. 1. The schedule of the ACR training and the treatments with electroconvulsive shock or drugs.



Treatments	ED50 (mA)
Saline	47.04
Spermine	41.28
DFMO	38.02
Aminoguanidine	35.20
Citicholine	33.07

Fig. 2. Probit plots of Log DOSE (mA: electrical current) and convulsive response in the treatments of CDP-choline, spermine, DFMO, and aminoguanidine.

Drug treatment & Electroconvulsive shock (ECS)

Each animal was intraperitoneally given for 10 consecutive days after the ACR training sessions with CDP-choline, 250 mg/kg (CC); spermine, 10 mg/kg (SM); dl-α-difluoromethylornithine, 250 mg /kg (DO); and aminoguanidine, 100 mg/kg (AG), respectively. And the electroconvulsive threshold (ECTh) was estimated by repeated current loading in a manner of the gradual increase from 31.62 mA via bilateral ear-clip electrodes using an Ugo-Basile ECT unit (set at 0.5 msec; 100 Hz; 1.5 sec). From the results, the ED_{50} of ECTh was calculated by probit plot analysis.

The control ED_{50} of saline treated rats was 47.04 mA, and the value was decreased by the 3 hr-pretreatment with CC, SM, DO, and AG, respectively, down to 33.07 mA, 41.28 mA, 38.02 mA, and 35.20 mA as shown in Fig. 2.

Therefore, single or repeated loading of electroconvulsive shock (ECS) was carried at 50 mA; 0.5 msec; 100 Hz; 1.5 sec. As shown in Fig. 1, the rats of the 5×ECS group were treated five times with single ECS per day on and after the 11th day of the ACR experiment with one day interval, and then on the 20th day, were tested for the ACR retention; those of the ECS-3h group were treated with single ECS administered at 3 hrs before the retention test; and those of the ECS-24h group were treated with single ECS at 24 hrs before the retention test.

Measurements of brain acetylcholine and polyamines

Rat brains were removed immediately after decapitation and then frozen in dry-ice powder. The frozen brains were stored below −80℃ for less than 6 days. From the tissue slices obtained from the brains using a Harvard template, the hypothalamus, hippocampus, and entorhinal cortex were, respectively, isolated by a punch technique

(Palkovits, 1980).

Brain acetylcholine analysis

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

Extraction and oxidation: Tissues of 10 mg or more were minced in 25 volumes of cold 5% trichloracetic acid (TCA), standed for 60 min, and spun out at 15,000×g for 5 min. The 100 ul of the supernatant was washed 5 times with 1.5 ml of chilled water-saturated diethyl ether for control of its pH up to 4 and was exposed to nitrogen gas stream for the removal of the residual ether. And then, 50 ul of the remained was added with 10 ul of 0.5% sodium metaperiodate to eliminate the reducing substances which interfere with the chemiluminescent reaction.

Luninescent measurement of ACh: The reaction medium were at first, consist of 0.4 ml of 200 mM sodium phosphate buffer, 15 ul of an assay chemiluminescent mixture and 10 ul of the extract, and finally added with 100 ul of acetylcholinesterase (AChE) reagent. And then the luminescent count were integrated for 60 sec using a Berthold Biolumat LB 2500C.

The recovery rate was about 72.1±8.4%. Just before the ACh determination, the assay luminescent mixture (250 ul) was prepared with 25 unit choline oxidase (100 ul DW), 10 unit HRP (50 ul DW) and 18 ug luminol (100 ul of 0.2 M Tris buffer, pH 8.6). And the AChE reagent were prepared as following: a 250 ul of AChE (1000 unit per ml) in cold DW was passed through a coarse Sephadex G-50 column of 5 ml volume equilibrated in DW, and the column was further eluted with 1.45 ml DW before collecting the enzyme eluate of about 0.7 ml, which was stored below −40℃. Immediately before the assay, the working reagent was prepared by dilution of the stock solution with 19 volumes of 0.2 M sodium phosphate buffer, pH 8.6.

Brain polyamine analysis

High performance liquid chromatography (HPLC) system: The system was consisted of a Knauer HPLC pump, a Rheodyne 7125 injection valve, an Erma ERC ODS-1161 column (3 um; 6× 100 mm), 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℃ according to the method of Choi *et al.*, (1988). Derivatization and HPLC analysis of polyamines were based upon the method of Spragg and Hutchings (1983). Tissues of 2 mg or more were homogenized with a glass homogenizer in 25 volumes of chilled 0.4 M perchloric acid containing 2 mM disodium EDTA and diaminooctane 50 ng as an internal standard. 100 ul of the homogenate was spun out at 15,00×g for 10 min, and 50 ul of the supernatant was evaporated by a Speed Vac (Sarvant) dryer, and the dry residue was dissolved in 100 ul of 1 M sodium carbonate, and then derivatized with 300 ul of FNBT reagent (a mixture of 10 ul FNBT and one ml of dimethyl sulfoxide) at 60℃ for 20 min. At the end of derivatization, 40 ul of 1 M histidine in 1 M sodium carbonate was added to the reaction mixture, and then derivatization continued for a further 5 min to scavenge excess FNBT. After cooling the mixture in a ice basket, the N-2'-nitro-4'-trifluoromethylphenyl derivatives of polyamines were extracted twice with 2 ml 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 1 ml of HPLC-grade methanol. 20 ul of the methanol solution was applied to the isocratic reversed phase HPLC analysis.

The polyamine derivatives were eluted with acetonitrile-DW (80: 20, v/v) solvent at flow rate of 1.2 ml/min within 20 min, showing the complete resolution. The eluent was monitored by a UV/VIS detector set at 242 nm equipped with 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.

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.

Data analysis

The results obtained from the ACR experiment were statistically analyzed by the Newman-Keuls test (Tallarida and Murray, 1987). And the other data were analyzed by the Student's T-test

RESULTS

ECS effect on ACR retention

The rats that did not reach the learning criteria of 10 or less ACRs of 30 trials per one session at the 10th training days, were considered impossible and therefore were excluded from the experiments. The remained rats displayed 2.0±0.8 ACRs on day 1 and then reached 12.5±4.3 ACRs on day 5 (not shown) and 13.7±1.0 ACRs on day 10, the last training day, respectively. Therefore, the training schedule of the 10 training days was applied in this study. In the retention test on the learned ACR behavior, that was carried out on the 10th day after the last training session, the number of ACRs was further increased upto 18.8 ±1.1 in the control rats treated with isotonic saline of 1.0 ml/kg/day (Fig. 3).

The increase of ACRs was slightly inhibited by single ECS administered 24 hr berfore the retention test (ECS-24h) but significantly suppressed by single ECS 3 hr berfore the test (ECS-3h) ($q_{3,24}$= 5.47, $p<0.01$) and repeated 5×ECS (one ECS/day with one day interval for 10 days) administered for 10 days (5×ECS) ($q_{4,24}$=7, 78, $p<0.01$). respectively (Fig. 3).

And the increase of ACRs obtained on the 10th day after the last training session was not affected by treatment with difluoromethylornithine (250 mg/kg/day: DO) or amioguanidine (10 mg/kg/day: AG) for 10 days but significantly inhibited by treatment with CDP-choline (250 mg/kg/day: CC) ($q_{4,30}$=7.79, $p<0.01$) or spermine (100 mg/kg/day: SM) ($q_{3,30}$=6.91, $p<0.01$) for 10 days (Fig. 4).

The ECS-3h induced interference ($q_{3,24}$=5.47, $p<$ 0.01) of ACR retention (Fig. 3) was not affected by CC, DO, but moderately worsened by AG, SM ($q_{4,28}$=4.23, $p<0.05$) (Fig. 3).

And the ACR retention, as shown in Fig. 6 was

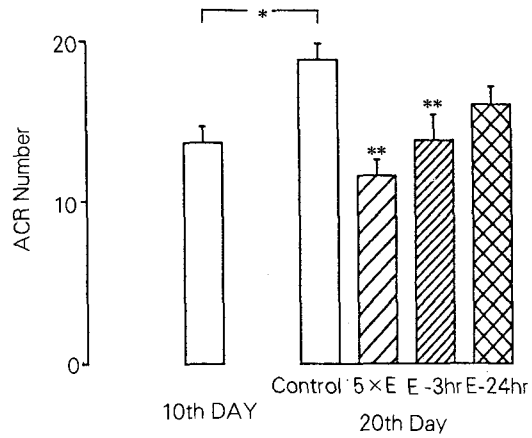


Fig. 3. Effects of various ECS treatments on the retension of active avoidance response. Each column indicates the mean±S.E., and * and ** mean $p<0.05$ and $p<0.01$, respectively.

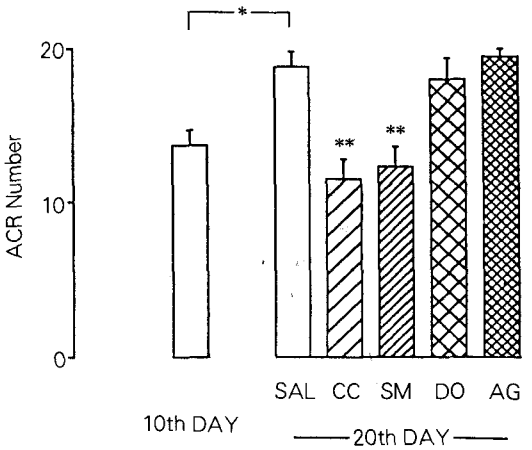


Fig. 4. Effects of CDP-choline (CC), spermine (SM), DFMO (DO), and aminoguanidine (AG) on the ACR-retension. Each coulumn indicates the mean ± S.E., and * and ** mean $p<$ 0.05 and $p<0.01$, respectively. And SAL means saline.

not changed by ECS-24h or a combined treatment of ECS-24h with one of the four drugs. But the 5× ECS induced impairment of ACR retention was significantly attenuated by CC ($q3.30=4.32$, $p<$ 0.05), SM ($q\ 4.30=6.14$, $p<0.01$), or AG ($q\ 5.30=$ 8.11, $p<0.01$) in the order of potency (Fig. 7).

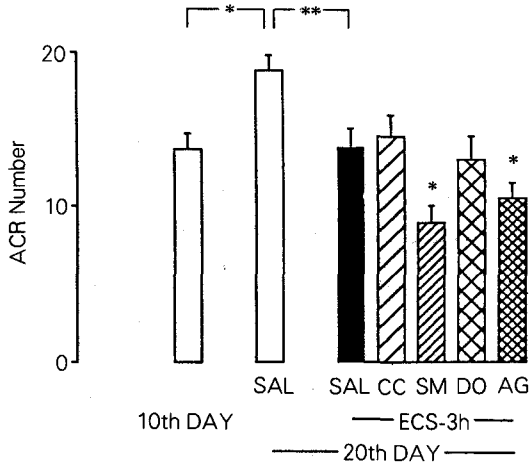


Fig. 5. Effects of CDP-choline, spermine, DFMO, and aminoguanidine on the acute suppressive effect of ECS on ACR-retension. Each column indicates the mean±S.E., and * and ** mean $p<0.05$ and $p<0.01$, respectively.

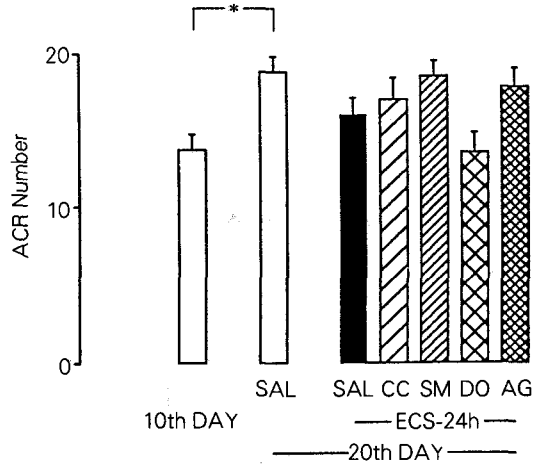


Fig. 6. Effects of CDP-choline, spermine, DFMO, and aminoguanidine on the change of ACR appeared 24hrs after ECS treatment. Each column indicates the mean±S.E., and * and ** mean $p<0.05$ and $p<0.01$, respectively.

ECS-induced change of brain acetylcholine content

The ACh contents of rat hypothalamus, hippocampus, and entorhinal cortex were estimated after 10 days-treatment with saline (control), CC, SM, DO, and AG, respectively.

As shown in Fig. 8-left A, B, C, the results revealed that the ACh contents of hypothalamus, hippocampus, and entorhinal cortex of the rats treated with saline were 125.5±16.3, 137.9±5.9, and 169.5±6.1 pmole/wet wt g, respectively.

These contents were not different from the ACh contents obtained 3 hr and 24 hr after single ECS. But they were significantly increased by treatment with CC or SM for 10 days and were not affected by the same treatment with DO or AG.

However, the ACh contents of all brain areas of SM-treated rats were gradually decreased for at least 24 hrs after single ECS, but the ACh of CC-treated rat brains, somewhat differently, showed the initial distinct decrease followed by the marked increase upto higher level than pre-ECS level.

And the brain ACh contents of rats treated with

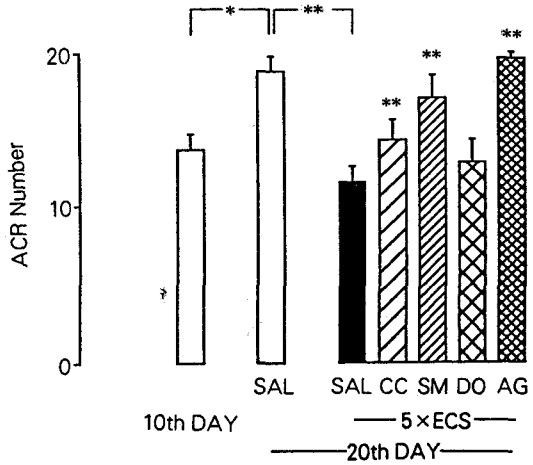


Fig. 7. Effects of CDP-choline, spermine, DFMO, and aminoguanidine on the suppression of ACR-retension induced by 5×ECS. Each column indicates the mean±S.E., and * and ** mean $p<0.05$ and $p<0.01$, respectively.

DO or AG for 10 days were gradually increased after single ECS upto statistically meaningful level, compared to that of control rats.

By the way, as shown in Fig. 8-right, repeated 5

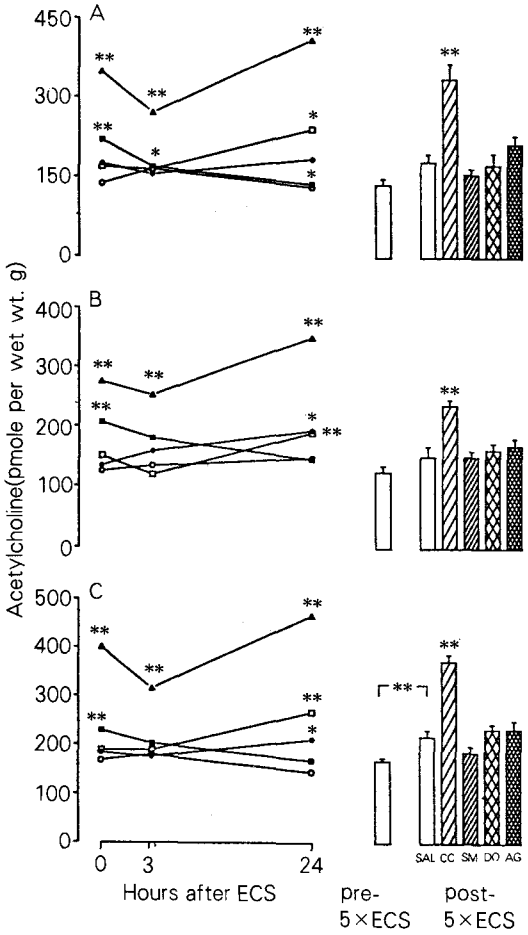


Fig. 8. Effects of CDP-choline, spermine, DFMO, and aminoguanidine on the ECS-induced change of the ACh content of the hypothalamus (A), hippocampus (B), and entorhinal cortex (C).

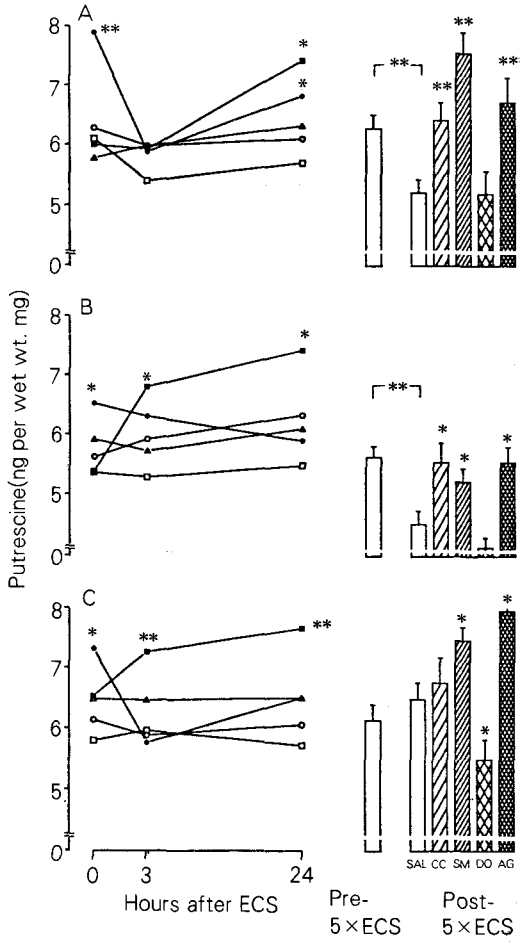


Fig. 9. Effects of CDP-choline, spermine, DFMO, and aminoguanidine on the ECS-induced change of the putrescine content of the hypothalamus (A), hippocampus (B), and entorhinal cortex (C).

×ECS regimen induced not only the slight increase of hypothalamus ACh but also the considerable increases of hippocampus and entorhinal cortex. And the the 5×ECS induced increase was moderately attenuated in the rats treated with SM but little affected by DO or AG. Needless to say, the brain ACh contents were markedly increased by combined of CC and 5×ECS.

ECS-induced changes of brain polyamine contents

The polyamine contents of rat hypothalamus (HT), hippocampus (HC), and entorhinal cortex (EC) were estimated after 10 days-treatment with saline (control), CC, SM, DO, and AG, respectively (Fig. 9, 10, 11), and the results, like the past report (Russell et al., 1974), showed that putrescine (Pt) was evenly distributed in rat brains, but the ratio of spermine (Sm)/spermidine (Sd) contents was higher in cortical region.

As a matter of fact, the brain Pt contents of HT, HC, and EC were 6.28±0.23, 5.62±0.19, and 6.14± 0.25 ug/wet wt g, respectively (Fig. 9); the Sd contents of HT, HC, and EC were 16.88±0.43, 13.56±

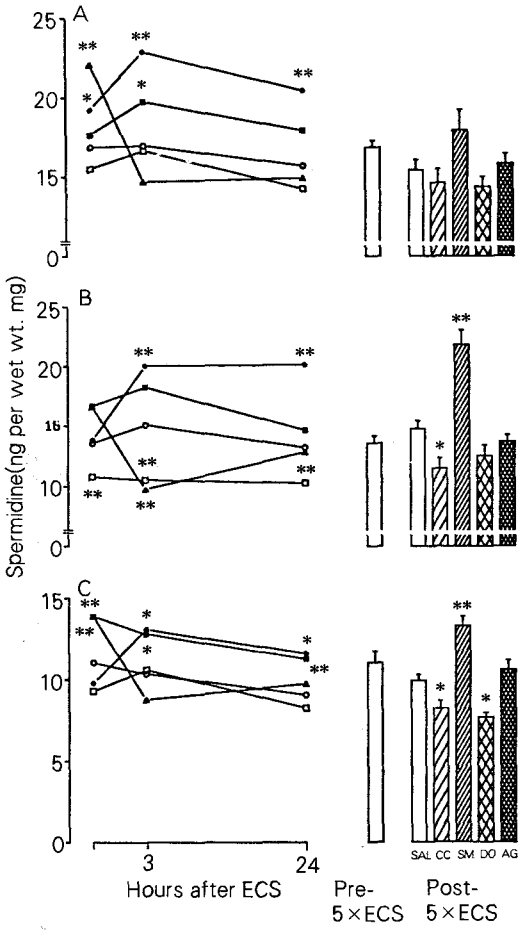


Fig. 10. Effects of CDP-choline, spermine, DFMO, and aminoguanidine on the ECS-induced change of the spermidine content of the hypothalamus (A), hippocampus (B), and entorhinal cortex (C).

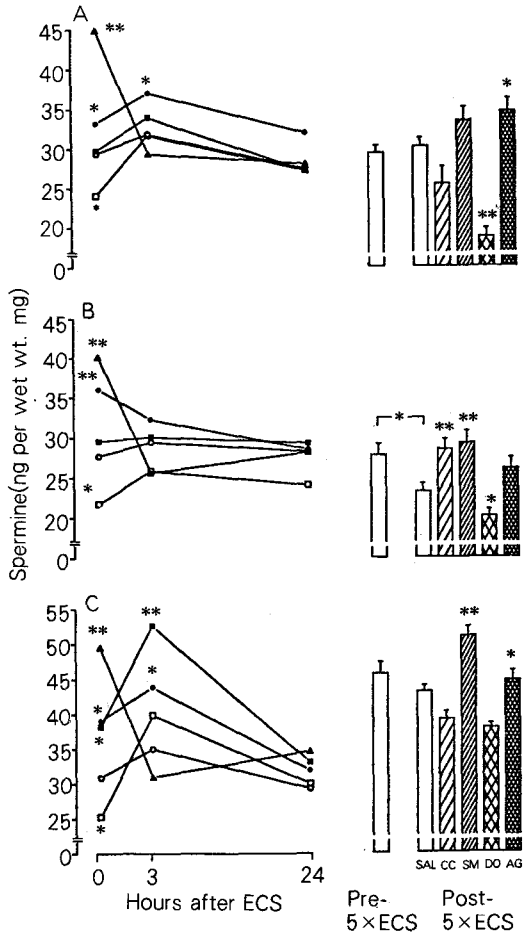


Fig. 11. Effects of CDP-choline, spermine, DFMO, and aminoguanidine on the ECS-induced change of the spermine content of the hypothalamus (A), hippocampus (B), and entorhinal cortex (C).

0.59, and 11.02±0.71 ug/wet wt g, respectively (Fig. 10); and the Sm contents of HT, HC, and EC were 29.36±0.91, 27.68±1.42, and 30.94±1.58 ug/wet wt g, respectively (Fig. 11). And single ECS did not significantly change these contents, but 5×ECS significantly decreased the Pt contents of HT and HC without meaningful changes of brain Sd and Sm contents (Fig. 9, 10, 11). The 5×ECS induced decrease of Pt content was accentuated by DO but completely blocked by CC, SM, or AG (Fig. 9-right).

The Pt contents of HT, HC, and EC were markedly increased by AG but little changed by CC, SM, or DO. But the contents of the rats treated with SM were gradually increased in HC and EC by 32.3% and 24.8%, respectively, in 24 hr after administration of single ECS (Fig. 9-A, B).

Unlike brain Pt contents, the Sd and Sm contents of HT, HC and EC, that were markedly increased by CC, showed the dramatic decreases by 3 hrs after single ECS (Fig. 10 & 11).

SM significantly increased the Sd content of HC and the Sd and Sm contents of EC without changes of those contents of HT, and the Sd of HC and

the Sm of EC were transiently increased after single ECS. DO significantly decreased the Sd of HC and the Sm of all the brain regions, and the decreased Sd content of HC was not affected by single ECS. But the Sm contents decreased by DO were recovered to control values or so after single ECS. And AG moderately increased the Sd content of HT and the Sm content of all the brain regions estimated in this study, and the polyamine contents increased by AG were further increased by 3 hrs after single ECS (Fig. 10 & 11).

By the way, the Sd and Sm contents were little changed by 5×ECS execpt the moderate decrease of HC-Sm content (Fig. 11), and the 5×ECS induced Sm decrease tend to be inhibited by AG, CC, and SM in the order of potency. And the Sd contents of HC and EC were more greatly decreased by combined treatment of CC and 5×ECS than by 5×ECS alone (Fig. 10).

However, in spite of 5×ECS, the Sm content of EC and the Sd contents of HC and EC were increased by SM, the Sd of EC and the Sm of HC were decreased by DO, and finally, the Sm content of HT and EC were significantly increased by AG (Fig. 10 & 11).

DISCUSSION

Extensive studies on the changes in learning and memory that occur during aging have been ongoing for over fifty years, but only a few informations are available concerning the mechanisms that could be responsible for these deficits.

So, pharmacotherapeutic approaches to cognitive disorders still remains a largely unsatisfactory undertaking (Goodnick and Gershon, 1983; Traub and Freedman, 1987).

However, recent progresses (Gibson *et al.*, 1987; McGaugh, 1989) in the researches on alterations of neurotransmissions underlying Alzheimer's disease and related dementias indicate that cholinergic systems are involved in memory storage (Bartus *et al.*, 1985; Davies, 1985). Furthermore, the formation of glucose derived neurotransmitters such as glutamate and γ-aminobutyrate (Gibson *et al.*, 1981) and dopamine (Finch, 1973) generally decrease with aging but to a lesser extent than ACh. And also in Alzheimer's disease, the reduction of cells occurs more markedly in the basal cholinergic systems than in other brain systems, including noradrenergic, dopaminergic, serotonergic, GABA-ergic, and somatostatin systems (Decker, 1987).

However, the precise role of decreased cholinergic function in the cognitive disorders that accompany aging is unclear (Gibson and Peterson, 1983) but may be related to the deprssion of Ca^{++}-dependent ACh release due to decreased neuronal uptake of Ca^{++} availability (Gibson *et al.*, 1981).

Gustafesson and Wigstrom (1988) found that the Ca^{++}-influx via the NMDA-receptor channel is critical for long-term potentiation (LTP) in hippocampus and the LTP requires depolarization of the postsynaptic cells coincident with activity in the presynaptic neuron.

By the way, ECS has been a widely used amnesia-inducing tool in rodents (McGaugh, 1966; Squirre, 1977; Lerer *et al.*, 1986), and scopolamine is also useful as an amnesia-inducer (Glick and Zimmerberg, 1972). As a matter of fact, ECS therapy that is superior to other therapeutic approaches on severe endogenous depression has the misfortune to induce cognitive impairments (Squire, 1977; Fink, 1987). And altough there are some rather conflicting reports related with the relation of brain ACh with seizure susceptibility (Karczmar, 1974) and ECS-induced changes of brain ACh concentration (Atterwill, 1984), many investigators agree with the cholinergic framework underlying epileptogenesis (Snead, 1983; Wasterlain *et al.*, 1986).

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

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

Polyamines activated the NMDA-receptors by

binding to an independent site of the NMDA-receptor (Reynolds, 1990), and α-difluoromethylornithine (DFMO: an irreversible inhibitor of ornithine decarboxylase, a rate limiting enzyme of polyamine synthesis), significantly reduced the brain damage produced by striatal injection of N-methyl-D-aspartate (Kish *et al.*, 1991).

Therefore, this study was undertaken to ascertain whether an acetylcholine precursor (CDP-choline) and the changes of brain polyamine contents induced by spermine, DFMO, or aminoguanidine (an irreversible inhibitor of copper-amine oxidase, particularly diamine oxidase) improve ECS-induced impairment of the retention of active conditioned avoidance response (ACR), with references of the changes of brain ACh and polyamine contents. As a result, the number of ACR obtained in the retention test that was carried out on the 10th day after the initial training session of 10 days, was further increased by about 37.2%, compared to that of the last training session.

The increase was not affected by single ECS loaded 24 hr before ACR test (ECS-24h) but significantly suppressed by single ECS loaded 3 hr before the test (ECS-3h) or ECSs loaded repeatedly five times (one ECS per day with one day interval for 10 days: 5×ECS). And the ACR increase was also suppressed by treatments with CDP-choline (250 mg/kg/day: CC) or spermine (100 mg/kg: SM) for 10 days but not affected by DFMO (250 mg/kg/day: DO) or aminoguanidine(100 mg/kg/day: AG). However, the CC or SM induced suppression was significantly attenuated by ECS-24h.

In contrast with ECS-24h, the ECS-3h induced suppression of ACR was little affected by CC, DO, or AG but more worsed by SM. And the marked suppression effect of 5×ECS on ACR-retention was significantly inhibibited by AG, SM, and CC in the order of inhibition magnitude.

These results suggests that the acute effect of ECS-3h on ACR-behavior seem to be rather related to other pathways than brain systems of ACh and polyamine metabolism, inferring those of ECS-24h and 5×ECS, that all of the brain ACh and polyamines have some beneficial potential in the improvement of ECS-induced ACR impairment, and finally that AG or its analogs may help promote the pharmacotherapeutic approaches to cognitive disorders. As a matter of course, several previous evidences defining that brain cholinergic system is profoundly involved in memory processing(Bartus *et al.*, 1985; Davies, 1985) and that polyamines, particularly spermine, inhibit the uptake by brain synaptosomes of ACh(Law *et al.*, 1984), seem to be closely related to the above results obtained in this study.

Therefore, the influences of ECS and four substances applied in this study on brain ACh and polyamines cotents were estimated to ascertain how they interact with each other in view of their metabolisms in discrete brain regions.

The brain contents of ACh and polyamines were little changed by 5×ECS as well as single ECS except marked 5×ECS induced decreases of the hypothalamus(HT) and hippocampus(HC) putrescine(Pt) contents and the HC-spermine(Sm) content. But the marked 5×ECS induced decreases of the HT-and HC-Pt contents and the HC-Sm content were not seen in rats treated with CC, SM, or AG, suggesting that 5×ECS induced impairment of ACR retention may be ascribed to the derangement of brain polyamine metabolism. In addition, (1) CC induced the significant increases of ACh, Sd, and Sm in HT, HC, and entorhinal cortex(EC) of rat brain; the brain Sd and Sm contents increased by CC were dramatically decreased by single ECS to the control level or somewhat lower; (2) SM induced the moderate increases of HC-Sd and EC-Sm contents as well as ACh contents all the brain areas, the Pt contents of HC and EC of rats treated with SM showed the significant and gradual increases by single ECS, and SM little affected the brain ACh content of 5×ECS group rats but significantly increased all of the brain polyamines, particularly Pt of HT and EC, Sd of HC and EC, and EC-Sm over the control level; (3) DO decreased HC-Sd, and Sm of all the brain areas, and the contents of EC-Sd, and Sm of all the areas measured were significantly lower in the rats treated in combination of DO and 5×ECS compared to that with 5×ECS alone; and finally, (4) AG little increased brain ACh content but induced the marked increase of brain Pt content and the moderate increases of HT-Sd and Sm of all the areas, and rats treated with combination of AG and 5×ECS showed the marked increase of

all the contents of polyamines.

Collectively taking these results into consideration, there are several meaningful interactions between acetylcholine or CDP-choline and polyamine metabolisms in the brain, and the interactions as well as the AG-effect unrelated with diamine oxidase may participate in improving of ECS-induced or age-related disturbances of memory functions.

By the way, the brain membrane phospholipids may be subtly changed by the experiences of learning, memory and epilepsy(Delgado-Escueta, 1980), and the phospholipid breakdown is induced in brain ischemia, electroshock, and so on (Bazan and Rodriguez de Turco, 1983). CDP-choline, a prinicipal intermediate of phosphatidylcholine synthesis, promotes clinical recovery in hemiplegia(Hazama *et al.*, 1980) and Parkinson's syndrome(Manaka *et al.*, 1974). And polyamines may be able to displace iron ions from phospholipid binding sites and inhibit lipid peroxidation under certain circumstances(Halliwell and Gutteridge, 1989). Polyamines play a role in the long-term adaptive response to electrical stimulation (Zawia *et al.*, 1990).

And there are recent evidences suggesting that aminoguanidine may have future therapeutic use in retinopathy(Hammes *et al.*, 1991), nephropathy (Hasegawa *et al.*, 1991), and peripheral neuropathy (Yagihashi *et al.*, 1992) of diabetes due to the inhibition of accumulation of advanced glycation end-products.

However, we have not found any report showing the direct effects of CDP-choline or polyamine related compounds for control of congnitive disorders, and furthermore, in the recent our experiment(in progress) on the learning of passive avoidance responce(PAR), the rats given by CC, SM, and DO at 3 hrs before a PAR training trial showed the significantly decreased latencies to cross into the dark compartment from the aversively bright one upon retesting 24 hrs later, but AG little affected the PAR latency as compared with that of the control rats. Therfore, the detailed studies on each of the following two problems: (1) the protective action of CC, SM, and AG, particularly AG from the ECS-impairment of ACR-retention and (2) the CDP-choline induced increase of brain Sd and Sm contents, may prove to be fruitful for the research of cognitive disorders.

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=국문초록=

백서의 조건회피반응-유지에 대한 경련성 전기충격의 저해작용에 미치는 CDP-Choline, Aminoguanidine, 및 Difluoromethylornithine의 영향에 관한 연구

: 뇌내 Acetylcholine과 Polyamine 함량-변동에 연관하여

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Two-way shuttle box에서 active conditioned response (ACR)을 10일간 매일 30회 부하하여 10회 이상 반응한 웅성 Wistar 백서를 이용하여, 경련성 전기충격 (ECS: 50 mA, 100 Hz, 1.5 sec)에 의한 ACR-유지의 변동에 미치는 CDP-choline (250 mg/kg/day/CC), aminoguanidine (100 mg/kg/day: AG), α-difluoromethylornithine (250 mg/kg/day, DO), 및 spermine (10/mg/kg/day: SM) 각각의 10일간 복강내-주사의 영향을 검토하였다.

그 결과, 10일간 획득된 ACR은 그 다음 10일후에 더욱 증가되었으며, 이는 일일 간격의 5회 ECS (5-ECS) 또는 ACR-유지검사 3시간전 일회 ECS (ECS-3h)로 현저히 억제되나, ACR-유지검사 24시간전의 일회 ECS (ECS-24h)로는 별 영향을 받지 않았다. 아울러, ACR의 자연증가 현상은 CC와 SM에 의하여 현저히 저하되었으나 AG와 DFMO에 의하여는 영향을 받지 않았다. 5-ECS에 의한 ACR-유지저해는 AG, SM, 및 CC에 의하여 유의하게 되었으며 DFMO에 의하여는 영향을 받지 않았다. ECS-3h 후의 ACR-유지저해는 SM와 AG에 의하여 다소 더 악화되었고 CC와 DFMO에 의하여는 별 영향을 받지 않았는데, 정상 백서에 비하여 큰 차를 보이지 않은 ECS-24h 후의 ACR은 CC, SM, DO, 및 AG에 의하여도 영향을 받지 않았다.

한편, ECS-3h와 ECS-24는 백서-대뇌 시상하부(HT), 해마(HC), 및 내후피질(EC)의 acetylcholine (ACh) 함량에 별 영향을 미치지 않았으나, 5×ECS는 다소 증가시켰으며 특히 EC의 ACh증가는 유의하였다. 아울러 CC과 SM도 대뇌-ACh 함량을 유의하게 증가시켰으나, DO와 AG는 별 영향을 미치지 않았다.

ECS-3h와 ECS-24h는 대뇌 HT, HC, 및 EC의 polyamine 함량에 영향을 미치지 않았으나, 5×ECS는 HT와 HC의 putrescine (Pt) 함량과 HC의 spermine (Sm) 함량을 각각 유의하게 감소시켰다. CC는 PT함량에 영향을 미치지 않았으나 spermidine (Sd)와 Sm 함량은 현저히 증가시켰다. 그러나 이같은 CC의 작용을 ECS-3 시간후와 24시간후에는 전혀 볼 수 없었을 뿐 아니라, ECS-3 시간후의 HC의 Sd 함량은 정상치보다 유의한 감소로 역전되었다. 또한 CC는 5×ECS에 의한 Pt와 Sm 함량-감소를 유의하게 억제하였다. SM은 전 부위의 Sd 함량과 EC의 Sm 함량을 유의하게 증가시켰고, 이같은 증가가 ECS-3 시간후에 더욱 상승되었으며, 이때 HC와 EC의 Pt함량도 유의하게 증가되었다. 또한 SM은 5×ECS에 의해 Pt와 Sm 함량-감소를 유의하게 억제하였다.

그러나 DO는 HC의 Sd와 모든 부위의 Sm 함량을 유의하게 감소시켰고 또한 Pt와 Sm에 대한 5×ECS의 감소작용을 더 상승시켰다. AG는 모든 부위의 Pt 함량을 현저히 증가시켰을 뿐 아니라 HT의 Sd와 모든 부위의 Sm도 유의하게 증가시켰고, 5×ECS에 의한 Pt와 Sm 함량-감소를 유의하게 억제하였다.

이상의 성적은 5×ECS로 나타나는 ACR의 유지-저해에 대한 aminoguanidine의 보호작용의 일부가 polyamine 대사에 대한 그의 diamine oxidase 억제작용에 기인됨을 시사하는 것으로 사료된다.