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Effect of Ketamine on the Acetylcholine Concentration of Various Regions of Rat Brain[1]

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= Abstract =After Ketamine administration (30 mg/kg, intraperitoneally), acetylcholine (ACh) concentrations did not change significantly at 10 min, but started to decrease in several brain regions at 30 min, and decreased markedly in all regions at 60 min. ACh levels returned to almost normal after 4 hrs. The decrease was most marked in pons-medulla, cerebral cortex, hippocampus, and corpus striatum. In these regions, a dose (5 mg/kg, intraperitoneally) far below the anesthetic doses decreased ACh levels as much as 30 mg/kg. The derease in ACh levels induced by ketamine may be associated with hallucination, delirium, dream, amnesia, and analgesia which occur in humans at subanesthetic doses of ketamine and during recovery from ketamine anesthesia.

Key words: ***Ketamine, Acetylcholine, Rat brain, Behavior***

INTRODUCTION

Ketamine is chemically related to phencyclidine (PCP), a hallucinogen, nicknamed angel dust, and was introduced as an anesthetic in 1965 (Dimino *et al.*, 1965). Although it has several merits as an anesthetic, the relatively frequent occurrence of post-anesthetic psychotic phenomena, such as hallucinations, deliriums or unpleasant dreams, severely limits its usefulness (Dundee and Wyant 1977). Clinically, these phenomena mimic the side effects of anticholinergics (Pigallo and Wingard 1979; Toro-Matos *et al.* 1980). In animal studies, ketamine has significant presynaptic and post-synaptic effects on the cholinergic system (Cohen *et al.* 1974; Amaki *et al.* 1978; Torda and Murphy 1979; Maleque *et al.* 1981; Aronstam *et al.* 1982). It is attempting to think that postanesthetic phenomena of ketamine may be closely related to the cholinergic system (Toro-Matos *et al.* 1980; Maleque *et al.* 1981; Aronstam *et al.* 1982). To study the possible relationships, we measured acetylcholine (ACh) concentrations until full recovery after an anesthetic dose; also after a subanesthetic dose considering that the postanesthetic phenomena also appear at subanesthetic doses, even as little as a 1/5 of anesthetic doses (Reier 1971).

MATERALS AND METHODS

Adult male Sprague-Dawley rats (260-300g) were kept at constant temperature (about 25°C) and housed for at least 7 days prior to study under a 12-h light, 12 h dark cycle. Food and water were allowed ad libitum. Ketamine hydrochloride (Ketalar) was a gift from Yuhan Corp., Seoul. Choline kinase (ATP: choline phosphotransferase; EC 2.7.1.32) and acetylcholinesterase (eel, type V; acetylcholine acetyl-hydrolose: EC 3.1.1.7) were purchased from Sigma Chemical; Sodium tetraphenylborate from Dojin Chemical. The [r-^{32}P] ATP (New England Nuclear Corp.) had an initial specific radioactivity of 43.0 Ci/mmol and was used through 1.5 half-lives. Bio-Rad AG 1 × 8 resin (200-400 mesh, formate form) was purchased from Bio-Rad Laboratories. All other chemicals were from commercial sources.

The animals were killed 10 min, 30 min, 1h, and

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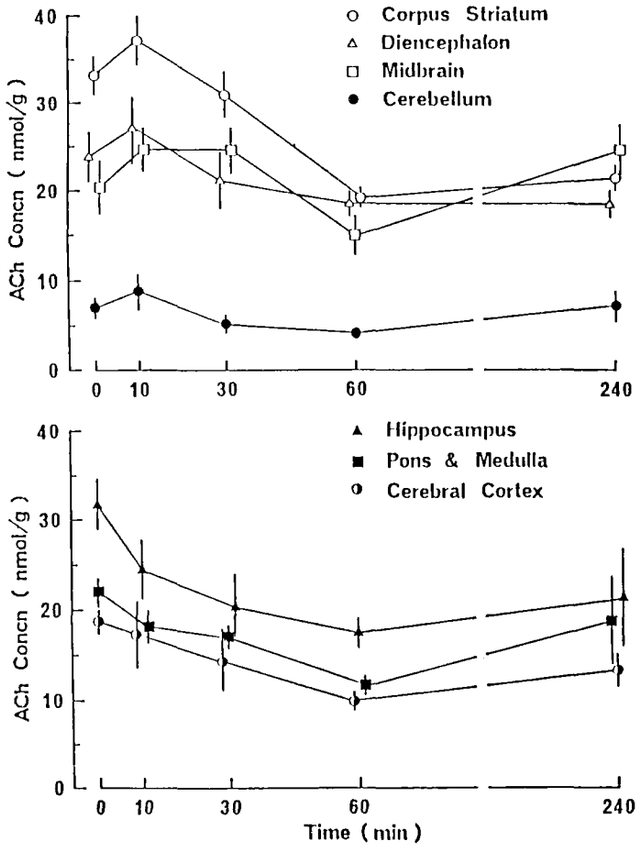

Fig. 1. Time course of the change of ACh concentrations after ketamine 30 mg/kg intraperitoneal administration. Values are means ± SEM of 7.4 experiments in average. Changes with time were statistically significant in corpus striatum, hippocampus, pons-medualla, and cerebral cortex ($P<0.001$ in corpus striatum: $P<0.05$ in hippocampus, pons-medulla, and cerebral cortex, not significant in other regions, by analysis of variance). In those regions, changes only at 60 min were statistically significant (*$P<0.05$; **$P<0.005$ as compared with the values at time 0, by q statistic).

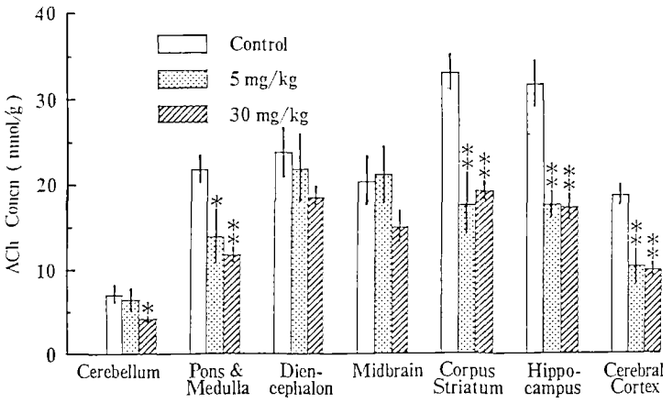

Fig. 2. ACh concentrations 60 minutes after ketamine 5 mg/kg and 30 mg/kg intraperitoneal administration. Values are means ± SEM of 8 experiments in average. *$P<0.05$; **$P<0.01$ as compared with control groups, by Student's t test. Values of 5 mg/kg group and values of 30 mg/kg group were not significantly different.

4h after a 30 mg/kg dose of ketamine HCl; 1h after a 5 mg/kg dose of ketamine HCl. Each dose of the drug (solution always freshly prepared by dissolving in distilled water) was injected intraperitoneally. Control groups received the same amounts of 0.9% physiological saline. To avoid effects of diurnal variations of ACh all animals were killed between 10:00 and 12:00 a.m. Immediately after decapitation, the brains were removed and separated into 7 regions (cerebellum, pons-medulla, diencephalon, midbrain, corpus striatum, hippocampus, and cerebral cortex), essentially as described by Glowinski and Iversen (1966). These procedures were performed on a dry ice plate to prevent hydrolysis of ACh. The dissected tissues were weighed immediately.

To measure ACh concentration, we used Goldberg and McCaman's radioenzymatic assay (1973). We used formic acid/acetone, tetra-phenylboron and HCl for extraction. The extract contains both the choline and ACh. The assay procedure uses a two stage assay. The first stage, in the presence of unlabelled ATP, results in the conversion of endogenous choline to unlabeled phosphorylcholine. In the second stage, by addition of acetylcholinesteras (AChE) and labeled ATP, ACh is simultaneously hydrolyzed and then phosphorylated. The labeled phosphorylcholine is then separated through barium precipitation and further by ion exchange chromatography. We did the procedure from decapitation to the 2nd incubation in a cold temperature to prevent postmortem changes in ACh levels due to hydrolysis by cholinesterases.

The results were analyzed using analysis of variance followed by q statistics (Fig. 1) and Student's t test (Fig. 2). A P value < 0.05 was considered significant.

RESULTS

Changes in ACh concentrations with time

Figure 1 shows changes in ACh concentrations with time, from anesthesia to full recovery. After ketamine administration, the ACh concentrations did not change significantly at 10 min, when the rats were anesthetized. However, there was a

Table 1. ACh concentration after ketamine 30 mg/kg i.p.administration (nmol/g)

Time(min)	Cerebellum	Pons & Med.	Diencephalon	Midbrain	Striatum	Hippocampus	Cerebrum
0	7.051 ±1.118	21.905 ±1.603	23.824 ±2.921	20.426 ±3.090	33.111 ±2.228	31.691 ±2.864	18.639 ±1.303
10	8.716 ±2.097	18.179 ±1.771	26.838 ±3.849	24.683 ±2.495	37.195 ±2.881	24.432 ±3.448	17.233 ±3.423
30	5.237 ±0.966	16.964 ±1.319	21.762 ±3.337	24.488 ±2.642	30.953 ±2.722	20.437 ±3.448	14.815 ±3.423
60	4.226 ±0.366	11.585* ±1.036	18.530 ±1.427	15.152 ±1.798	19.177** ±1.117	17.355* ±1.704	9.742* ±1.004
240	7.326 ±1.622	18.886 ±4.880	18.360 ±1.869	24.601 ±3.139	21.386 ±1.448	21.366 ±5.458	13.327 ±1.724
p	NS	.01<p<.05	NS	NS	p<.001	.01<p<.05	.01<p<.05

The data represent mean ± S.E.M of 7.4 experiments in average.
p values were calculated by analysis of variance and q statistic.
*.01 < p < .05, ** .001 < p < .005 as compared with the value at time 0.

Table 2. ACh concentration 60 minutes after ketamine 5 mg/kg & 30 mg/kg i.p. administration (nmol/g)

Dose(mg/kg)	Cerebellum	Pons & Med.	Diencephalon	Midbrain	Striatum	Hippocampus	Cerebrum
0	7.051 ±1.118	21.905 ±1.603	23.824 ±2.921	20.426 ±3.090	33.111 ±2.228	31.691 ±2.864	18.689 ±1.303
5	6.370 ±1.448	13.935* ±3.249	21.958 ±4.002	21.200 ±3.374	18.120** ±3.660	17.554** ±1.597	10.527** ±2.078
30	4.226* ±0.366	11.585** ±1.036	18.530 ±1.427	15.152 ±1.798	19.177** ±1.117	17.355** ±1.704	9.742** ±1.004

The data represent mean ± S.E.M. of 8 experiments in average.
p values were calculated by Student's t test.
* .01 < p < .05, ** p < .01 as compared with control group.
Values of 5 mg/kg group & values of 30 mg/kg group were not significantly different.

tendency to decrease at 30 min. At 60 min when all rats had recovered from anesthesia, the ACh concentrations decreased markedly in the pons, medulla, cerebral cortex, hippocampus, and corpus striatum, by 42 to 47 percent. Also in other regions, the decrease was most marked at 60 min, although statistically not significant. The ACh levels returned to almost normal after 4h. The degree of return was relatively small in the corpus striatum, hippocampus, and cerebral cortex (see also Table 1).

Changes in ACh concentrations with different doses.

Fig. 2 shows changes in the ACh levels with different doses. An hour after a 5 mg/kg ketamine administration, there were marked decreases in the corpus striatum, hippocampus, cerebral cortex, and pons-medulla, by 36 to 45 percent. Both a subanesthetic and an anesthetic doses showed significant decreases in the same regions and also by the same degrees (see also Table 2).

DISCUSSION

Although we killed rats by decapitation instead of microwave irradiation, our control data were similar to others obtained in microwaved animals (Nagai *et al.* 1978; Khandelwal *et al.* 1981). No change in

ACh levels at 10 min postinjection were observed, as has been reported by others (Cohen *et al.* 1974; Nagai *et al.* 1978). However, the decrease in ACh levels at 30, 60, 240 min postinjection and at a low dose could not be compared to others, since such studies have been lacking.

Even a very low dose of ketamine caused a significant decrease in ACh concentrations in our study. This might be a result of a decrease in ACh synthesis and/or an increase in hydrolysis of ACh. In addition, we should consider the possibility of an increase in ACh release, since ACh is rapidly hydrolyzed as soon as it is released. However, ketamine does not influence ACh release (Torda and Murphy 1979; Cronnelly *et al.* 1973) or even decrease it *in vitro* (Amaki *et al.* 1978). *In vivo,* there's a possibility that increase in cholinergic neuron activity (and hence increase in ACh release) may lead to a decrease of ACh concentrations (Guyenet *et al.* 1975), but ketamine's effect on ACh release *in vivo* is not significant either (Sanfacon and Labreque 1982). On AChE which catalyzes hydrolysis of ACh, ketamine has inhibitory action, (Cohen *et al.* 1974; Amaki *et al.* 1978) which will rather increase ACh concentration. Apparently, the mechanim of the decrease in the ACh concentration by ketamine does not seem to have its effect on the ACh release or hydrolysis. Among factors that control ACh synthesis, the amount of choline carried to the brain by blood flow, may be increased by ketamine, because ketamine at usual anesthetic doses increases cerebral blood flow up to 80 percent (Dundee and Wyant 1977). Therefore, the cause of the decrease in the ACh concentration rnay be ketamine's inhibitory effect on choline reuptake or choline acetyltransferase. However, studies about the possiblility have been lacking.

Our data are not incompatible with a possible relationship between the decrease in the ACh concentration by ketamine in rats and hallucination, delirium, and dream by ketamine in humans. First of all, both occur at similar time sequences and with a dose far below the anesthetic doses. Furthermore, both an anesthetic and a subanesthetic dose showed significant decreases in the same regions and also by the same degrees. In addition, these psychic phenomena are known to be closely related to the cholinergic system, (Paster *et al.* 1974; Lipowski 1980; Silberman *et al.* 1980; Cooper *et al.* 1982; Hong 1983) and ketamine seems to have a greater and more direct effect on the cholinergic system than on the monoamine neurotransmitter systems (Sung *et al.* 1973; Biggio *et al.* 1974; Glisson *et al.* 1976; Ylitalo *et al.* 1976; Kari *et al.* 1978). Similar reasoning may be applied also to analgesia and amnesia caused by ketamine (Dundee and Wyant 1977; Cooper *et al.* 1982). However it is difficult to consider mechanisms of various psychic reactions by ketamine only with the change in ACh levels. Concentration does not reflect activity of cholinergic system as much as the turnover rate and no change in concentration does not necessarily mean no change in turnover rate. We should also consider the possibility that the main mechanism of psychic reactions induced by ketamine is its postsynaptic action (Maleque *et al.* 1981; Aronstam *et al.* 1982). Changes in ACh levels reflect presynaptic events rather than postsynaptic events. Ketamine may have greater postsynaptic actions (Maleque *et al.* 1981; Crounelly *et al.* 1973). Therefore, further studies are required to elucidate the mechanism of the psychic reactions by ketamine.

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= 국문초록 =

Ketamine이 백서뇌 부위별 Acetylcholine 함량에 미치는 영향

서울대학교 의과대학 정신과학교실 및 약리학교실*

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복강내 Ketamine 투여 (30 mg/kg) 후 백서뇌 acetylcholine 농도는 10분 후 변화가 없었으나 30분 후 일부 부위에서 감소하여 60분 후에는 모든 부위에서 현저하게 감소하였다. 가장 현저한 감소를 보인 부위는 뇌교, 연수, 대뇌피질, 해마, 선조체였으며, 이들 부위에서는 마취용량에 훨씬 미달하는 용량 (5 mg/kg)으로 30 mg/kg 용량에서와 같은 acetylcholine 농도 감소를 보였다.

이상의 실험결과로 ketamine에 의한 뇌 acetylcholine 농도 감소가 마취용량 이하의 ketamine이나 ketamine 마취 회복기에 나타나는 환각, 망상, 꿈, 기억상실 및 진통과 유관하리라 시사된다.