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Effect of Anesthetics on Phosphate (^{32}P) Metabolism of the Rat Brain ^{*1}

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The effect of chlorpromazine (Chp), ether, hexobarbital (evipal) and urethan on the content and turnover rate of phosphate compounds in rat brain tissue was investigated. ^{32}P was taken up into various phosphate fractions in the following order of decreasing rate: ATP, creatine phosphate (CP), nucleoprotein (NP) and phospholipid (LP). ATP concentration was increased and the turnover rate of the fraction was diminished by Chp with evipal (Chp-evipal) and Chp with urethan (Chp-urethan) anesthesia. CP concentration was not appreciably changed but its turnover rate was decreased except for Chp with ether (Chp-ether) anesthesia in which a reduction of CP and acceleration of its turnover rate was noted. These findings suggest that the utilization of high energy phosphate esters is inhibited by Chp-evipal and Chp-urethan but not by Chp-ether. The incorporation of ^{32}P into LP and NP was significantly decreased after 2-hour anesthesia, while it was rather increased within 30 minutes particularly with Chp-ether anesthesia. A different mode of action of Chp-ether on phosphate metabolism of the brain is suspected when compared with that of Chp-evipal as well as Chp-urethan.

The significance of phosphate metabolism in the brain activity has been the subject of controversy. Recent evidence has been presented that the incorporation of ^{32}P into organic phosphate esters in the brain is more rapid than in the muscle (LINDBERG and ERNSTER¹⁰), and a constant renewal of phosphates occurs even in the fraction of phospholipid as well as nucleoprotein of the brain tissue (CHAIKOFF⁹).

The possibility that the content and turnover rate of phosphate compounds may be changed by depression of the central nervous system offers pharmacological interest in studies on the mode of action of anesthetic drugs. While it has been reported that the metabolism of inorganic phosphate and organic esters are affected by barbiturates (STONE¹³, LE PAGE⁹), NAKA¹¹), KOZAWA⁸) and chlorpromazine (GRENELL⁶, GRISWALD⁷, OGAWA¹²), precise information on the turnover rate of various phosphate fractions is still lacking.

In the present experiments the phosphate compounds of the brain in rats were analyzed and a turnover rate of ^{32}P in each fraction was determined simultaneously. The values of controls were statistically compared with those of rats anesthetized with chlorpromazine (Chp), ether, hexobarbital (evipal) and urethan.

METHODS

White rats of either sex weighing from 150 to 200 g were divided into five

^{*1} Reported in *Folia pharmacol. jap.* 55 : 1430 (1959) in Japanese.

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groups. The rats of the first group were not anesthetized and served as controls. The second group was given 20 mg/kg of Chp alone intramuscularly. The third, fourth and fifth one receiving ether, evipal or urethan combined with Chp referred to the group of Chp-ether, Chp-evipal and Chp-urethan, respectively. The radioisotope solution containing about 20 μ c of ^{32}P was injected into the subarachnoid space of the rat brain by the method described by LINDBERG and ERNSTER¹⁰. When convulsion, paralysis or other severe lesions were encountered, the animals were rejected.

In order to prevent post-mortem decomposition of organic phosphate compounds, particularly labile phosphate esters, liquid air was employed for freezing the tissue. The head of the animal was drowned in liquid air and frozen within a few seconds. The frozen brain was removed and weighed quickly. Under continuous cooling with the use of an ice-salt mixture, the brain was taken into a mortar and ground with 10 cc of 10 per cent trichloroacetic acid solution. After standing for about 10 minutes, the suspension was centrifuged at 2500 rpm by means of refrigerated centrifuge. The supernatant solution refers to an acid soluble phosphate (TP) fraction. An aliquot was transferred to the centrifuge tube and then a magnesia mixture was added. After standing overnight in a refrigerator, the precipitate containing inorganic phosphate (IP) fraction was removed. The supernatant solution was adjusted to 0.2N by HNO_3 , ammonium molybdate was added and left standing for 1.5 hours in a refrigerator to obtain the precipitate containing the creatine phosphate (CP) fraction. The supernatant solution adjusted to 0.5N by HNO_3 was boiled for 15 minutes and readjusted to 0.2N by NH_4OH . After standing with the ammonium molybdate solution, the precipitate containing the ATP fraction was removed.

A phospholipid (LP) fraction was extracted from the residue by refluxing twice with 10 cc of 80 per cent alcohol and then three times with an alcohol-ether mixture boiled gently. The ultimate residue refers to the nucleoprotein (NP) fraction.

Each organic phosphate fraction was placed in a small Kjeldahl flask and brought into solution by digestion with 10N H_2SO_4 and a few drops of H_2O_2 . An aliquot was analyzed for phosphate by the method of FISKE and SUBBAROW¹¹.

Radioactivity was measured using a Geiger-Müller counter. The specific activity was given as the counts per minute of ^{32}P divided by the number of mg of phosphate. The relative specific activity indicating the turnover rate of a fraction was represented by a ratio ($\times 1000$) of its specific activity to that of the precursor. Inorganic phosphate was taken as the precursor of creatine phosphate as well as ATP, and the total acid soluble phosphate was of phospholipid and nucleoprotein.

RESULTS

1. Control

The rats of this group were not anesthetized but 0.1 cc of ^{32}P solution was injected into the subarachnoid space. The analysis of the brain was carried out 30 minutes as well as 2 hours after administration of the radioisotope solution.

Table 1 shows the phosphate values of IP, CP, ATP, LP and NP fraction. There was no significant difference between the 30-minute and the 2-hour experiment. The findings indicate that phosphate metabolism of the brain was not affected by injecting ^{32}P solution.

The relative specific activity of ^{32}P in IP, CP, ATP, LP and NP fraction was given in table 2. The renewal rate of phosphate fraction was shown in the following order of decreasing rate: ATP, CP, NP and LP. The renewal rate of ATP

TABLE 1. Phosphorous Content of the Phosphate Fractions in Rat Brain Tissue

Drug	Time	IP mg%	CP mg%	ATP mg%	TP mg%	LP mg%	NP mg%
Control	30min	* 30.2±15.3(7) †	6.7± 2.6(7)	26.3± 5.8(7)	85.2± 8.5(7)	196.8±33.7(7)	72.0±17.4(7)
	2 hrs	31.4±12.4(7)	4.3± 1.4(7)	23.2± 7.0(7)	89.8±10.2(7)	174.9±30.3(7)	72.1±16.5(7)
Chlorpromazine	30min	32.3±11.4(6)	7.7± 6.2(6)	27.7± 6.9(6)	86.3±11.6(6)	192.2±24.9(6)	71.4±11.6(6)
	2 hrs	34.9± 9.1(7)	4.6± 1.6(6)	24.4± 7.2(7)	89.9± 6.3(7)	191.5±15.5(7)	71.0± 9.6(7)
Chp-ether	30min	33.2± 5.7(4)	1.8± 0.4(4)	26.3± 3.8(4)	80.7± 9.0(7)	195.9±11.6(7)	56.3±10.6(7)
	2 hrs	26.0± 3.6(4)	2.7± 0.7(3)	30.9± 3.6(4)	72.4±12.1(4)	185.5±17.1(4)	59.5± 1.1(4)
Chp-evipal	30min	28.5± 2.5(4)	3.6± 0.5(4)	33.8± 7.8(4)	85.7± 7.5(7)	194.6±16.8(7)	61.5± 2.2(4)
	2 hrs	37.1± 8.5(4)	7.8± 1.1(4)	33.4± 6.6(4)	85.3± 5.0(4)	186.7±22.5(4)	57.5± 5.7(4)
Chp-urethan	30min	38.1± 7.4(4)	4.0± 0.7(4)	34.9±14.0(4)	84.8± 5.1(4)	193.0±20.4(4)	61.5± 9.1(4)
	2 hrs	26.8± 5.1(4)	6.0± 1.1(3)	34.3± 3.5(4)	81.5± 4.5(4)	190.3± 9.0(4)	58.8± 5.5(4)

* Values expressed mean $\pm \sqrt{\frac{\sum x_i^2 - N\bar{x}^2}{N-1}}$ † Numbers in parenthesis are number of animals employed.

TABLE 2. Relative Specific Activity of the Phosphate Fractions in Rat Brain Tissue

Drug	Time	CP	ATP	LP	NP
Control	30min	* 252.6±185.4(6) †	777.6±451.7(6)	31.4±11.3(7)	154.5±42.0(7)
	2 hrs	460.7±100.6(5)	838.1±121.9(7)	70.5±23.4(7)	279.3±72.0(7)
Chlorpromazine	30min	228.9±170.7(6)	462.8±324.1(6)	27.4± 7.9(6)	130.5±34.8(6)
	2 hrs	263.5± 98.1(5)	849.0±465.5(7)	38.1± 8.1(7)	187.5±47.3(7)
Chp-ether	30min	1182.0± 555.3(4)	717.2± 91.6(4)	46.8±23.9(7)	327.2±67.0(7)
	2 hrs	1349.3±1053.8(3)	859.0±255.2(4)	37.5± 2.9(4)	199.9±42.7(4)
Chp-evipal	30min	138.4± 39.6(4)	168.8± 57.0(4)	49.3±15.6(7)	80.5±22.6(4)
	2 hrs	78.2± 43.1(4)	134.2± 53.3(4)	35.2± 6.1(4)	205.9±29.4(4)
Chp-urethan	30min	196.4± 149.6(4)	193.1±134.2(4)	10.5± 1.6(4)	134.5±55.0(4)
	2 hrs	216.7± 147.0(3)	250.6± 21.9(4)	14.0± 4.4(4)	92.1±18.8(4)

* Values expressed mean $\pm \sqrt{\frac{\sum x_i^2 - N\bar{x}^2}{N-1}}$ † Numbers in parenthesis are number of animals employed.

attained 80 per cent within 30 minutes and very gradual increase occurred thereafter. Other fractions showed rather slow renewal rate within 30 minutes but attained twice after 2 hours.

2. *Effect of Chp*

Prior to the administration of ^{32}P solution, the rats were given 20 mg/kg of Chp intramuscularly. The phosphate values of IP, CP, ATP, LP and NP fraction are shown in table 1. When compared with the controls the phosphate levels in the experimental group showed no appreciable change.

The relative specific activity is presented in table 2. It was found that ATP in the 30-minute and CP in the 2-hour experiment were significantly ($P < 0.05$) lower than the controls. The decrease of LP and NP in the 2-hour experiment was also statistically significant ($P < 0.01$).

3. *Effect of Chp-ether anesthesia*

The stage of anesthesia was maintained approximately at surgical level by controlling the amount of ether applied to the animal. As can be seen in table 1, the phosphate level of the CP and ATP fractions in the 30-minute experiment was significantly lower ($P < 0.05$) than in the controls as well as in the rats receiving Chp alone. Contrary to the above fraction, the phosphate level of ATP was significantly higher ($P < 0.05$) than in the controls and Chp-treated rats.

While there was no appreciable change in the relative specific activity of ATP, that of CP was diminished ($P < 0.01$) in the 30-minute as well as in the 2-hour experiment. In LP and NP fractions the relative specific activities were elevated within 30 minutes but after 2 hours they were decreased significantly ($P < 0.01$).

4. *Effect of Chp-evipal anesthesia*

In this experiment the animal was given 50 mg/kg of evipal combined with Chp. Table 1 shows the phosphate level and table 2, the relative specific activity of each fraction. The phosphate level of IP, CP and LP fractions were not significantly altered. An increase of phosphate level in the ATP fraction was significant compared to the controls ($P < 0.01$) and the Chp-treated rats ($P < 0.05$). In the NP fraction a significant ($P < 0.05$) decrease was noted.

The relative specific activity of the ATP fraction was smaller than the controls in the 30-minute ($P < 0.05$) as well as in the 2-hour experiment ($P < 0.01$), that of the CP fraction also being lowered in the 2-hour experiment. While the relative specific activity of the LP fraction was higher ($P < 0.05$) within 30 minutes, after 2 hours it was significantly ($P < 0.01$) reduced.

5. *Effect of Chp-urethan anesthesia*

After the Chp was given, additional injection of 1.0 g/kg of urethan was made intramuscularly. Table 1 shows that the phosphate level of the IP, CP and LP fractions was not appreciably changed. There was, however, a significant increase ($P < 0.01$) of ATP fraction in the 2-hour experiment. The phosphate level of the NP fraction was lower ($P < 0.05$) than the controls as well as the Chp-treated animals.

The relative specific activity of ATP ($P < 0.01$) and NP ($P < 0.05$) fractions in the 30-minute experiment and that of the CP ($P < 0.05$), ATP ($P < 0.01$), LP ($P < 0.01$) and NP ($P < 0.01$) fractions in the 2-hour experiment were significantly decreased.

DISCUSSION

An accumulation of high energy phosphate esters may be expected when brain

activity is depressed by anesthetic agents, the utilization of these esters being inhibited. It holds true that an accumulation of a fraction in the metabolic pathway results in a reduction of its renewal rate. The evidence derived from the present experiments, that ATP fraction is increased and that the turnover rate of the fraction is significantly reduced, appears to be in favor of this view.

The findings reported by GRENNELL⁶⁾ and KOZAWA⁸⁾ that IP fraction is decreased while CP fraction is increased by Chp and barbiturates, are not confirmed in the present studies. It is evident that the turnover rate of this fraction is reduced by Chp-*evipal* and Chp-*urethan* anesthesia. Unlike these anesthetics, Chp-*ether* produced a characteristic change in the CP fraction. This was somewhat decreased and the renewal rate was augmented. This fact may suggest that the utilization of the fraction is accelerated by Chp-*ether* anesthesia.

As emphasized by BRODY²⁾ the concentration of energy-rich compounds in the brain is the net result of the generation and utilization of these compounds. A reduced renewal rate represents not only an inhibited utilization but also generation of the fraction. The changes in the content and renewal rate of these compounds are not necessarily the same under anesthesia but probably depend upon anesthetic agents employed.

Although the phosphate level of the LP fraction was not appreciably affected, the renewal rate of the fraction appears to be increased during the early stage of Chp-*ether* and Chp-*urethan* anesthesia. However, after 2 hours the incorporation of ³²P into this fraction is uniformly reduced by all anesthetics employed. The findings are in agreement with those reported by WASE *et al.*¹⁴ and DAWSON and RICHTER⁴⁾. The turnover rate of the NP fraction was significantly decreased except in the case of Chp-*ether* anesthesia in which an increase was observed in the 30-minute experiment. These results indicate that anesthetic drugs affect not only the metabolism of high-energy phosphate esters but also of structural phospholipid and nucleoprotein (ABOOD¹⁾). In this respect Chp-*ether* shows a quite different mode of action compared to that of Chp-*evipal* and Chp-*urethan*.

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