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ON THE TURNOVER OF ACETYLCHOLINE IN MOUSE BRAIN: INFLUENCE OF DOSE SIZE OF DEUTERIUM LABELLED CHOLINE GIVEN AS PRECURSOR

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Abstract—The influence of the dose size of precursor choline (Ch) on the turnover rate of acetylcholine (ACh) has been studied in whole brain and striatum of mice. Different doses of deuterium labelled Ch (d_6-Ch) were injected i.v. and concentrations of endogenous and d_6-labelled Ch and ACh were estimated at various times by mass fragmentography using deuterium labelled (d_9-) Ch and ACh as internal standards. At doses of 1.25–20 μmole/kg of d_6-Ch the endogenous concentration of Ch in blood was transiently elevated for 5 min. However, the endogenous concentrations of Ch and ACh in whole brain and striatum were not affected. The fractional rate constant of ACh synthesis and efflux, K_a, a measure of the turnover rate of ACh, was influenced by the dose size of precursor d_6-Ch. In whole brain, K_a was *ca.* 0.7 at doses of 5–20 μmole/kg of d_6-Ch. At lower doses K_a increased to *ca.* 1.0. In striatum, K_a was *ca.* 0.7 at doses of 20–40 μmole/kg of the precursor. At lower doses K_a became smaller and decreased to 0.10 at a dose of 1.25 μmole/kg of d_6-Ch. When the calculations of K_a were corrected for the part of d_6-Ch concentration in the brain contained in blood the figures were invariably increased by 20–40%. However, the increase was not correlated with the precursor dose size. It was found that the ratio between S_{ACh} and S_{Ch} decreased with increasing dose of d_6-Ch until it reached plateau values at doses of 2.5 μmole/kg and higher in whole brain and at 10–20 μmole/kg and higher in striatum. It is proposed that the decreased ratio at low concentrations of d_6-Ch depends on saturation of the high affinity (HA) uptake system for Ch coupled to ACh synthesis. At higher doses of d_6-Ch, ACh synthesis by the low affinity (LA) uptake system for Ch became increasingly important and was not yet saturated at the highest dose given (40 μmole/kg). It is suggested that the synthesis rate of ACh can be studied with either very low doses of labelled Ch that do not saturate the HA uptake system or with sufficiently high doses at which the influence of the LA uptake system on the synthesis rate of ACh is dominant.

Several methods have been used in the attempt to assess the *in vivo* turnover rate of acetylcholine (ACh) in the brain of rats and mice. Many of these methods use an isotopically labelled precursor of ACh, e.g. choline (Ch). Ch has been given i.v. as such [1] or as phosphorylcholine [2] either as a pulse dose or as an infusion for several minutes. The turnover rate may also be measured after injection of an irreversible cholinesterase inhibitor, after which the increase of the endogenous level of ACh in a certain time is measured [3]. Similarly, the decrease of ACh with time may be measured after blockade of ACh synthesis with intracerebro ventricularly injected hemicholinium-3 [4]. The turnover values obtained depend among other factors on measurements of correct endogenous concentrations of ACh and Ch. It has recently been possible to achieve this by the use of focused microwave irradiation to kill the animals. With this technique, the enzymes responsible for the postmortem changes of ACh and Ch levels are effectively destroyed.

It has been emphasized that a necessary prerequisite to obtain correct turnover values is to carry out the estimate at steady-state levels of Ch in blood and brain. This has been achieved in different ways. Eckernäs *et al.* [5] infused unlabelled Ch until a new steady state was obtained (15 min) whereafter they continued the infusion with labelled Ch and measured its incorporation into ACh. Costa and co-workers [6] used infusion of labelled phosphorylcholine which liberates Ch in the blood.

In experiments where labelled Ch is given and the turnover rate is calculated from the specific activity curves of Ch and ACh, dubious results may be obtained because the measured specific activities of Ch and ACh may not be representative of the pool in which ACh is synthesized. An underestimation of the specific activity of Ch will result in too high turnover rates and vice versa. This would be the case if the Ch concentration in cholinergic neurons is different from other brain structures. Another problem in this context can arise from co-estimation of labelled Ch which is contained in the blood. In the case of ACh it is now well established that it occurs in at least two pools, a cytoplasmic and a vesicle pool. Furthermore, it is widely believed that only the former pool, the so-called free pool of ACh, is directly involved in synthesis and release of the transmitter. As it represents only about half of the whole concentration of ACh in the neuron another ambiquity is introduced in the estimation of turnover, since the whole pool is used in the expression of the specific activity of ACh.

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To evaluate how precursor Ch may affect the calculation of the ACh turnover rate, mice were injected with different doses of labelled Ch and labelled and endogenous concentrations of Ch and ACh were measured in blood, whole brain and striatum. The mice were killed by focused microwave irradiation, and concentrations of labelled and endogenous ACh and Ch were measured by a method [7] which employs mass fragmentography.

MATERIALS AND METHODS

Animals and drugs. Male NMR, albino mice weighing 20–27 g were used. *N*-(2-hydroxyethyl)-*N*,*N*,*N*-tri-[$^2H_2$, ^1H]methylammonium iodide (d_6-Ch) was used as precursor to ACh.

N - (2 - hydroxyethyl) - *N*,*N*,*N* - tri - [$^2H_3$]methyl - ammonium iodide (d_9-Ch) and *N*-(2-acetoxyethyl)-*N*,*N*,*N*-tri-[$^2H_3$]methylammonium iodide (d_9-ACh) were used as internal standards. *N*-(2-acetoxyethyl)-*N*,*N*,*N*-tri-[$^2H_2$, ^1H]methylammonium iodide (d_6-ACh) was used for calibration purposes. The syntheses of the deuterium labelled compounds have been described previously [7].

Procedure. The precursor d_6-Ch was given during 1 sec in the tail vein in doses of 1.25, 2.5, 5, 10, 20 and 40 μmole/kg in saline in a volume of 5 ml/kg. The mice were killed and the blood and brain were analysed for labelled and unlabelled Ch and ACh.

Estimation of ACh and Ch in brain. The mice were killed by focused microwave irradiation on the head with 2.5 kW for 0.68 sec (Metabostate, Gerling-Moore, Palo Alto, CA). The whole brains were removed and homogenized directly in 4 ml 0.4 M $HClO_4$ or striata from 1–2 mice were excised, weighed, pooled and homogenized in 2 ml 0.4 M $HClO_4$ with an Ultra-Turrax homogenizer in a plastic scintillation flask (25 ml).

After addition of internal standards (whole brain: 0.25 nmole d_9-ACh and 0.60 nmole d_9-Ch; striata: 0.10 nmole d_9-ACh and 0.30 nmole d_9-Ch) the homogenates were left for 20 min at +4° and then centrifuged for 20 min at 100,000 *g* and +4°. Endogenous ACh and Ch together with their deuterated variants were extracted with dipicrylamine in dichloromethane as ion pairs. The Ch moieties were derivatized with propionyl chloride and the resulting mixture of ACh and propionyl choline derivatives was demethylated with sodium thiophenoxide and analysed by mass fragmentography according to Karlén *et al.* [7].

Estimation of Ch in blood. The mice were killed by a blow on the head, after which their throats were cut. About 0.2–0.5 g blood was collected into 5 ml saline in 10 ml heparinized centrifuge tubes. After centrifugation at 3000 rev/min for 10 min the diluted plasma (about 4 ml) was transferred to a 25 ml centrifuge tube. After addition of d_9-Ch (10 nmole in 1.0 ml) and 2.5 ml 1.2 M $HClO_4$ the samples were centrifuged at 100,000 *g* for 20 min. The supernatant was then analysed in the same way as described for brain samples. The results are expressed as nmole/g whole blood.

Calculation of turnover rate of ACh. The turnover of ACh (TR) was obtained from the specific activity curves of ACh (S_{ACh}) and Ch (S_{Ch}) between 15 and 45 sec after the injection of d_6-Ch, according to Zilversmit [8]. The following equation, where the subscripts 1 and 2 refer to 15 and 45 sec, respectively, was used to calculate the fractional rate constant, K_a, of ACh efflux.

$$K_a = \frac{2(S_{ACh_2} - S_{ACh_1})}{\Delta t(S_{Ch_1} - S_{ACh_1} + S_{Ch_2} - S_{ACh_2})}.$$

TR could then be calculated by multiplying K_a with the endogenous concentration of ACh. To obtain a K_a-value modified to account for the presence of blood in brain, the d_6-Ch used was obtained by subtracting the blood content of the d_6-Ch from the measured value. The assumed content of blood in brain is 1.9% in cortex and 1.1% in striatum [9].

RESULTS

The blood concentrations of d_6-Ch in mice injected with different doses are depicted in Fig. 1, the concentrations of Ch, ACh and their deuterated analogues in whole brain in Table 1 and those in striatum in Table 2. When plotting the blood concentration of d_6-Ch obtained after doses of 1.25–10 μmole/kg semilogarithmically vs time, parallel and rectilinear curves were obtained between 45 sec and 5 min with half-lives of about 2.2 min (Fig. 1). Similarly, when plotting the brain concentrations of d_6-Ch vs time, linear and parallel curves were obtained from 15 sec in animals given doses from 1.25 to 5 μmole/kg (Fig. 2). At 20 μmole/kg the curve from 2.5 min was parallel with those of animals given the lower doses. The half-lives of these curves were about 2.4 min, e.g. roughly the same as those of blood. The brain concentrations of d_6-Ch in whole brain and striatum after a dose of 20 μmole/kg have been followed for 20 and 60 min, respectively (Fig. 3). The half-lives in this experiment were 8.5 and 54 min.

Injection of d_6-Ch caused an increase of the concentration of endogenous Ch (13.9 ± 1.6 nmole/g, mean ± S.D.) in blood at 15 sec to about the same level (16–20 nmole/g) regardless of the dose size.

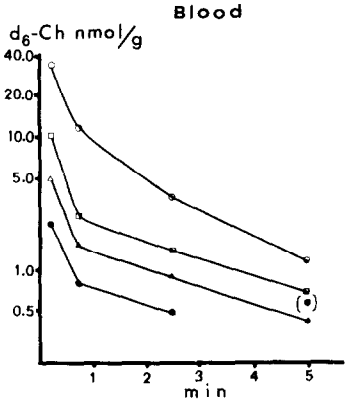

Fig. 1. Blood concentration time curves of d_6-Ch in mice injected i.v. with 1.25 (●——●), 2.5 (△——△), 5 (□——□) and 10 (○——○) μmole/kg d_6-Ch.

Table 1. Concentration (nmole/g) of endogenous and d_6-substituted ACh and Ch in whole brain of mice injected i.v. with different doses of d_6-Ch (mean ± S.D.)

d_6-Ch dose (μmole/kg)	No. of animals	Time after inj. (min)	ACh	d_6-ACh	Ch	d_6-Ch
	16	0.25	22.3 ± 3.0	0.08 ± 0.02	31.8 ± 5.0	0.36 ± 0.08
	12	0.75	25.7 ± 3.7	0.16 ± 0.04	33.6 ± 4.6	0.28 ± 0.07
1.25	4	1.5	20.8 ± 3.4	0.14 ± 0.02	40.7 ± 1.0	0.21 ± 0.03
	4	2.5	20.5 ± 2.4	0.13 ± 0.04	40.3 ± 2.0	0.16 ± 0.05
	4	5.0	20.7 ± 1.5	0.08 ± 0.01	32.1 ± 1.1	0.09 ± 0.01
	8	0.25	23.0 ± 2.4	0.12 ± 0.03	31.9 ± 7.5	0.62 ± 0.15
	10	0.75	23.8 ± 3.3	0.25 ± 0.04	33.4 ± 3.8	0.54 ± 0.05
2.5	4	1.5	27.1 ± 1.2	0.26 ± 0.04	38.9 ± 7.2	0.39 ± 0.02
	4	2.5	22.7 ± 2.6	0.20 ± 0.02	33.3 ± 4.1	0.25 ± 0.04
	4	5.0	20.2 ± 4.5	0.12 ± 0.03	31.5 ± 3.3	0.13 ± 0.03
	4	0.25	25.9 ± 3.9	0.14 ± 0.02	26.7 ± 5.3	0.86 ± 0.10
	4	0.75	22.4 ± 1.8	0.25 ± 0.02	34.2 ± 0.7	0.72 ± 0.08
5.0	4	1.5	28.9 ± 2.6	0.47 ± 0.07	32.8 ± 1.9	0.65 ± 0.14
	4	2.5	24.0 ± 1.0	0.35 ± 0.07	36.4 ± 3.5	0.46 ± 0.01
	6	5.0	24.3 ± 2.8	0.20 ± 0.04	37.2 ± 5.2	0.27 ± 0.05
	7	0.25	24.2 ± 2.3	0.20 ± 0.04	34.0 ± 3.4	3.59 ± 1.09
20.0	7	0.75	24.8 ± 4.4	0.75 ± 0.21	32.6 ± 8.1	2.34 ± 0.54
	9	2.5	23.6 ± 2.9	0.80 ± 0.20	33.6 ± 7.1	0.90 ± 0.23
	4	5.0	24.5 ± 3.3	0.73 ± 0.10	28.5 ± 1.5	0.47 ± 0.10

Table 2. Concentration (nmole/g) of endogenous and d_6-substituted ACh and Ch in striatum of mice injected with different doses of d_6-Ch (mean ± S.D.)

d_6-Ch dose (μmole/kg)	No. of animals	Time after inj. (min)	ACh	d_6-ACh	Ch	d_6-Ch
2.5	10	0.25	73.4 ± 14.6	0.51 ± 0.19	65.3 ± 21.3	0.63 ± 0.18
	11	0.75	84.0 ± 10.8	0.59 ± 0.22	59.9 ± 17.3	0.48 ± 0.12
5	3	0.25	71.1 ± 8.2	0.36 ± 0.02	49.3 ± 6.5	1.01 ± 0.03
	3	0.75	72.4 ± 11.7	0.61 ± 0.07	45.6 ± 5.3	0.50 ± 0.09
10	5	0.25	85.5 ± 10.2	0.55 ± 0.07	59.2 ± 7.9	2.76 ± 0.67
	3	0.75	87.4 ± 5.4	1.04 ± 0.07	43.6 ± 7.7	0.92 ± 0.11
20	6	0.25	92.9 ± 9.1	0.64 ± 0.24	67.7 ± 28.7	4.32 ± 2.00
	12	0.75	69.6 ± 9.2	1.43 ± 0.38	45.2 ± 10.2	2.19 ± 0.47
40	4	0.25	72.2 ± 9.0	0.80 ± 0.13	51.5 ± 4.7	5.88 ± 1.20
	4	0.75	71.3 ± 13.8	2.45 ± 0.29	47.9 ± 4.4	3.22 ± 0.55

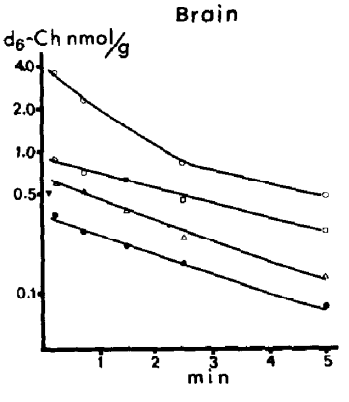

Fig. 2. Whole brain concentration time curves of d_6-Ch in mice injected i.v. with 1.25 (●——●), 2.5 (△——△), 5 (□——□) and 10 (○——○) μmole/kg d_6-Ch.

The increase was significant ($P < 0.01$) only after doses of 5 μmole/kg and higher. The level was normalized after 5 min. The endogenous levels of Ch in whole brain and striatum were, however, unaffected by the increased blood concentration (Tables 1 and 2). Neither were the endogenous levels of ACh in whole brain and striatum affected.

The first part of the specific activity time curves of ACh and Ch in whole brain after i.v. injection of d_6-Ch are depicted in Fig. 4. The S_{ACh}-curve is linear from 0 to 45 sec at doses of 5 μmole/kg and above. By increasing the dose of d_6-Ch, the S_{Ch}-curve becomes increasingly steeper and the area between the S_{Ch}- and S_{ACh}-curves also becomes larger. Since the area is the denominator in the Zilversmit equation, the K_a-value will be affected to a great degree. However, the increase in S_{ACh} is balanced by the increase in area between the curves, and K_a reaches

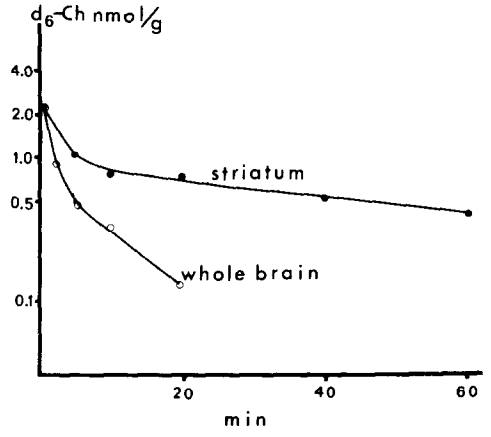

Fig. 3. Whole brain and striatum concentration time curves of d_6-Ch in mice injected i.v. with 20 μmole/kg d_6-Ch.

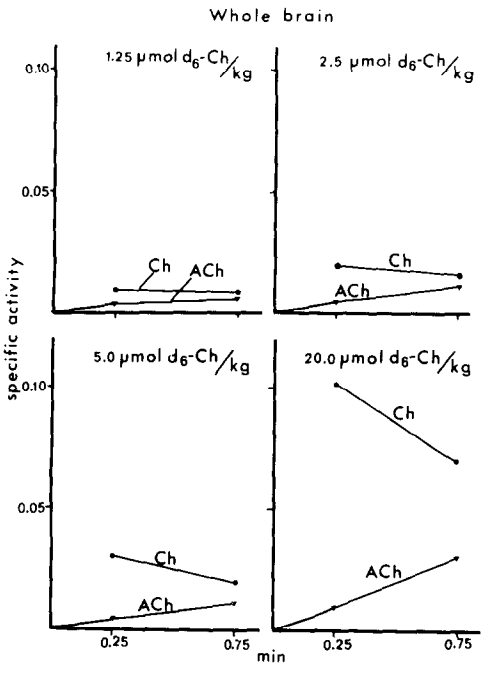

Fig. 4. Whole brain specific activity time curves of Ch and ACh in mice injected i.v. with 1.25, 2.5, 5 and 10 μmole/kg d_6-Ch.

a plateau level with increasing doses of precursor (Table 3).

In the striatum the general picture is different than in the whole brain. The S_{ACh}-curves become linear first at higher doses of d_6-Ch. At 2.5 μmole/kg, or less, no increase, or a very small increase, in S_{ACh} (between 15 and 45 sec) was noted (Fig. 5). Since

Table 3. Specific activities of ACh and Ch and K_a-values in whole brain obtained after different doses of d_6-Ch

d_6-Ch dose (μmole/kg)	Time (min)	S_{ACh}	S_{Ch}	$S_{Ch\ (corr)}$	K_a	$K_{a\ (corr)}$
1.25	0.25	0.0036	0.0110	0.0097	0.98	1.16
	0.75	0.0060	0.0084	0.0082		
2.5	0.25	0.0050	0.0191	0.0163	1.10	1.36
	0.75	0.0076	0.0159	0.0150		
5	0.25	0.0055	0.0312	0.0245	0.63	0.81
	0.75	0.0110	0.0205	0.0192		
20	0.25	0.0082	0.0955	0.0798	0.68	0.82
	0.75	0.0294	0.0670	0.0610		

K_a (min^{-1}) was calculated by the Zilversmit equation from S_{ACh} and S_{Ch} at 0.25 and 0.75 min. The corrected figures $S_{Ch\ (corr)}$ and $K_{a\ (corr)}$ were obtained by subtracting d_6-Ch contained in cerebral blood.

Table 4. Specific activities of ACh and Ch and K_a-values in striatum obtained after different doses of d_6-Ch

d_6-Ch dose (μmole/kg)	Time (min)	S_{ACh}	S_{Ch}	$S_{Ch\ (corr)}$	K_a	$K_{a\ (corr)}$
2.5	0.25	0.0069	0.0096	0.0088	0.11	0.16
	0.75	0.0070	0.0079	0.0076		
5	0.25	0.0068	0.0200	0.0178	0.38	0.46
	0.75	0.0083	0.0109	0.0103		
10	0.25	0.0065	0.0451	–	0.45	–
	0.75	0.0117	0.0194	–		
20	0.25	0.0069	0.0602	0.0551	0.63	0.75
	0.75	0.0201	0.0463	0.0435		
40	0.25	0.0110	0.1024	–	0.73	–
	0.75	0.0332	0.0629	–		

K_a (min^{-1}) was calculated by the Zilversmit equation from S_{ACh} and S_{Ch} at 0.25 and 0.75 min. The corrected figures $S_{Ch\ (corr)}$ and $K_{a\ (corr)}$ were obtained by subtracting d_6-Ch contained in cerebral blood.

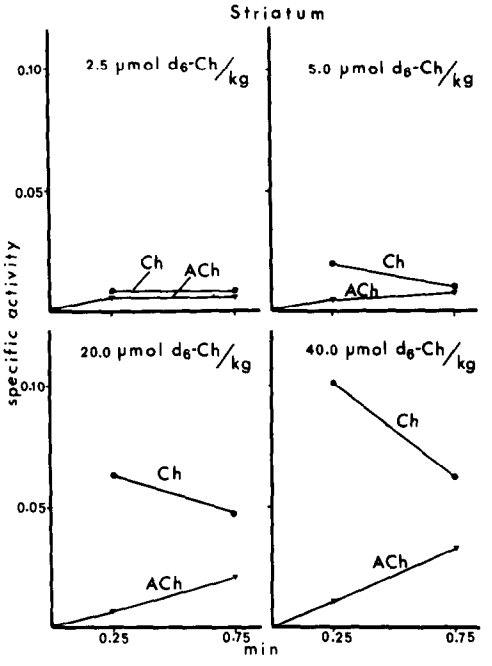

Fig. 5. Striatum specific activity time curves of Ch and ACh in mice injected i.v. with 2.5, 5, 20 and 40 μmole/kg d_6-Ch.

K_a is calculated from the increase of S_{ACh} between 15 and 45 sec, the value approaches zero at these low doses (Table 4). Also the precursor dose size seems to affect K_a in the opposite way to whole brain since at doses below 10 μmole/kg the apparent expression for K_a becomes smaller and approaches zero. Thus, the values of K_a in whole brain and striatum seems to fall in two or more categories (Table 3 and 4). At doses of 5 μmole/kg and above in whole brain and 20 μmole/kg and above in striatum the K_a-values were *ca.* 0.7. In whole brain the values become larger by about 60% when lowering the dose, while in striatum the values become smaller and approach zero when the dose is lowered. Characteristic for the values around 0.7 is that in these experiments the slope of the S_{ACh}-curve is linear up to 45 sec.

We have also studied the influence on the calculation of K_a of d_6-Ch in unequilibrated blood within the cerebral blood vessels. When S_{Ch}, used in the calculation of K_a, was adjusted for d_6-Ch contained in blood, the K_a-value increased with 20–40% at the different doses of d_6-Ch (Tables 3 and 4).

To obtain the same S_{Ch} in striatum as in whole brain, the dose of the precursor had to be doubled. Since the d_6-Ch concentrations were about the same in both pools, the explanation for this is that the endogenous Ch levels are about twice as high in striatum as in whole brain.

DISCUSSION

The aim of the present investigation was to study the effect of the size of the precursor dose on the turnover rate of ACh. After pulse injection of d_6-Ch at doses up to 5 μmole/kg the concentration of the precursor in the blood after a rapid mixing phase declined logarithmically for the first 5 min with a half-life of 2.2 min.

Aquilonius and Eckernäs [9] found a biphasic decline in blood in rat after [^{3}H]-Ch with half-lives of 1.7 and 11 min, respectively, for the two slopes. Since the blood concentrations were followed for only 5 min the second phase of the curve is not seen.

However, when following the brain concentration of d_6-Ch for 20 min a second phase with a half-life of about 8 min is seen (Fig. 3). Since the elimination curves in brain are parallel with those in the blood (Figs 1 and 2), it is concluded that the second phase with a half-life of 11 min seen in brain between 5 and 20 min (Fig. 3) can be inferred to also exist in blood (as shown in the rat by Aquilonius and Eckernäs [9]). The biphasic decline indicates that the Ch kinetics are according to a model with at least two compartments. At doses of 1.25, 2.5 and 5 μmole/kg, the apparent volumes of distribution of d_6-Ch ($V\beta$) were calculated to be 1250, 1250 and 1470 ml/kg, respectively. Aquilonius and Eckernäs [9] found 1520 ml/kg for the rat. The apparent volumes of distribution in whole brain and striatum were similar since the β-phases of the two curves intercept at about the same concentration at zero time (Fig. 3).

There is, however, a difference in Ch metabolism between the larger brain structures as reflected by whole brain and striatum since the terminal elimination phase is longer (54 min) in this brain structure.

It is shown that the endogenous levels of Ch and ACh are not affected by the increased blood levels of Ch caused by injection of d_6-Ch, thus demonstrating that steady-state levels are maintained in brain even after large precursor doses.

The K_a-values in whole brain, which at doses of 5 μmole/kg and above are *ca.* 0.7, became larger (1.0) when smaller precursor doses were used. When the values were corrected for extra-cerebral d_6-Ch contained in blood, the figures were increased (Table 3). However, the increase in K_a was not dose related and was lower for the highest dose of d_6-Ch.

In striatum, about the same K_a-values (0.7) as in whole brain were obtained at precursor doses of 20 μmole/kg or higher. At lower doses the K_a-values became smaller with decreasing dose. This is in contrast to results obtained by Nordberg [10], who obtained K_a-values of about 0.7 in striatum after pulse injection of doses as small as 7 nmole ^{3}H-Ch.

The main finding in this study is that the size of the tracer dose influences the shape of the specific activity curve of precursor and product and consequently the calculated K_a-values, especially in the striatum. This was unexpected since it is believed that the tracer would mix uniformly in the precursor pool utilized for the synthesis of the product. The slope of the specific activity curve of ACh is a measure of the rate of incorporation of d_6-Ch into ACh and may be assessed by the ratio between S_{ACh} and S_{Ch}. These ratios plotted against the dose of d_6-Ch approached constant values at doses of 2.5 μmole/kg and higher in whole brain and at doses of 10–20 μmole/kg and higher in striatum (Fig. 6). At lower doses the ratios increased. One explanation might

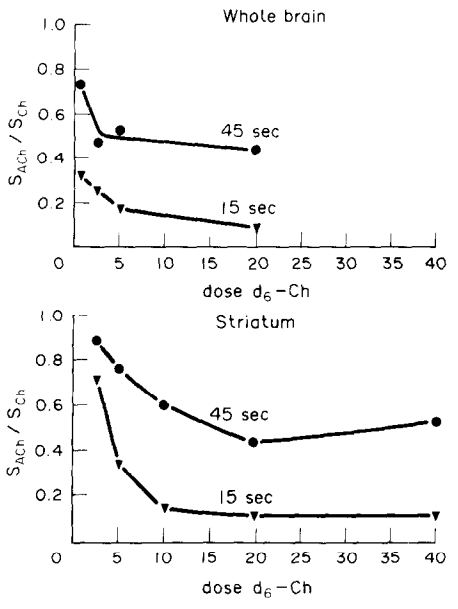

Fig. 6. Ratio between specific activities of ACh (S_{ACh}) and Ch (S_{Ch}) 15 and 45 sec after different precursor doses of d_6-Ch in whole brain and striatum.

be that part of d_6-Ch is taken up and contained in tissues not connected with the Ch-pool used for synthesis of ACh. However, in this case the ratio would never approach a constant value. Therefore, this explanation does not seem valid. Another explanation might be that Ch is taken up in the neuron by two mechanisms, a sodium-dependent high affinity (HA) uptake (K_m 1–10 μM) with a limited capacity and a low affinity (LA) uptake (K_m 50–100 μM) with a high capacity [11, 12]. While there is considerable evidence that the rate-limiting step in ACh synthesis in nondepolarized tissue is a sodium-dependent HA transport system for Ch [11–13], Jope and Jenden [14] have shown that ACh synthesis can be inhibited without altering the HA uptake system. In a study by Ksiezak-Reding and Goldberg [15] it was shown that by omitting chloride in a rat brain synaptosomal system, only the LA kinetic component of the uptake system could be detected. In spite of this, synaptosomes could still synthesize ACh (30–60% of control) under the nondepolarizing conditions. Thus, both the HA and the LA systems may be operative in the synthesis of ACh.

It can be hypothesized that the HA uptake system is playing an increasingly greater role for ACh synthesis compared to the LA uptake system when decreasing the dose. The decrease in ratio with increasing tracer dose would depend on saturation of the HA uptake system. In increasing the dose above 5 μmole/kg in whole brain and 10–20 μmole/kg in striatum the LA uptake system would be increasingly important and only the influence of this system on ACh synthesis is detected.

In striatum the same S_{ACh} (~0.0070) was obtained 15 sec after injection of precursor at doses up to 20 μmole/kg, although S_{Ch} increased 6-fold, indicating that the HA uptake system is rapidly saturated (Table 4).

When giving tracer doses below those given in this study a new constant ratio between S_{ACh} and S_{Ch} will probably be reached. In this hypothetical region below the saturation concentration of the HA uptake system its influence on the synthesis rate of ACh is probably studied. In support of this supposition Nordberg and Sundwall [16] reported an increase in S_{ACh} of about 35% between 30 and 60 sec in mouse striatum after a pulse injection of 10 ng [^{3}H]choline. In this experiment they found a K_a-value of about 0.7, i.e. the same as we find when using a dose of 400 nmole (20 μmole/kg). This tends to show that the turnover of ACh can be studied with either low tracer doses and an unsaturated HA uptake system or at higher doses when the LA uptake system is dominant. In the former case sensitive analytical techniques using radioactive tracer of high specific activity must be used. However, when using deuterium labelled tracer with a less sensitive but specific technique, doses high enough for the LA uptake system must be used. In this case the steady-state level of endogenous Ch and the surplus tracer Ch contained in blood has to be considered.

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