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CHARACTERIZATION OF ENZYMATIC ACETYLCHOLINE SYNTHESIS BY MOUSE BRAIN, RAT SPERM, AND PURIFIED CARNITINE ACETYLTRANSFERASE*

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Abstract—Enzymatic acetylcholine synthesis by mouse brain, rat sperm, and purified pigeon breast muscle carnitine acetyltransferase was monitored. Optimal assay procedure, choline substrate requirements, the extent of acetylcholine synthesis, and the effects of acetyltransferase inhibitors were investigated to determine if acetylcholine in rat sperm is synthesized by choline acetyltransferase or by another enzyme that utilizes choline as a substrate. Of two assay procedures tested, the liquid cation-exchange procedure utilizing a butyronitrile–tetraphenylboron extraction was judged superior to an anion-exchange resin procedure. The latter procedure gave falsely high acetylcholine synthesis readings due to another acetylated contaminant (probably acetylcarnitine). The K_m for choline substrate in acetylcholine syntheses by mouse brain, which is a source of choline acetyltransferase, was 0.623 mM choline; this was 300 times less than the choline substrate K_m with rat sperm (207 mM choline) and 80 times less than the K_m with purified carnitine acetyltransferase (50.6 mM choline). Rat sperm had a V_{max} [3718 pmoles AcCh·min^{-1}·(mg protein)$^{-1}$] that was >2-fold that of mouse brain [1603 pmoles AcCh·min^{-1}·(mg protein)$^{-1}$]. A specific inhibitor of choline acetyltransferase, 4-(1--naphthylvinyl)pyridine (500 μM), abolished acetylcholine synthesis by mouse brain, but it caused only a 52 per cent inhibition of acetylcholine synthesis by rat sperm and only a 12 per cent inhibition of acetylcholine synthesis by purified carnitine acetyltransferase. These data indicate that an enzyme other than classical or "true" choline acetyltransferase is responsible for acetylcholine synthesis by rat sperm. Because of the high content of carnitine acetyltransferase in rat sperm and the ability of carnitine acetyltransferase from pigeon breast muscle to synthesize acetylcholine, carnitine acetyltransferase is the most probable enzyme responsible for acetylcholine synthesis in rat sperm.

The existence in spermatozoa of a choline acetyltransferase – acetylcholine – acetylcholinesterase system that is responsible for the regulation of motility has been proposed [1–5]. Nascent acetylcholine (AcCh§) has been reported in rat, human, rabbit, and bull spermatozoa [4, 5]. Choline acetyltransferase (choline acetylase; acetyl-CoA: choline-*O*-acetyltransferase, EC 2.3.1.6; ChAc) has been reported in human, bull, rat, rabbit, and ram spermatozoa [4–6]. Doubt has been expressed as to the establishment of true ChAc in spermatozoa [7]. Two major sources of error have been noted in determining ChAc activity. The first source of error is a contaminant, most notably acetylcarnitine (AcCar) [7] causing spurious AcCh formation. The second source of error is true AcCh formed by carnitine acetyltransferase (carnitine acetylase; acetyl-CoA: carnitine-*O*-acetyltransferase, EC 2.3.1.7; CarAc) that has utilized choline as a poor substrate for carnitine [7]. The most common procedures used to determine ChAc are an anion-exchange resin based assay [8, 9] and a liquid cation-exchange based assay [10–12]. The anion-exchange resin assay measures radiolabeled AcCh formed by incubation of choline with radiolabeled acetyl-CoA. The radiolabeled AcCh is quantitated by passing the incubation mixture over an anion-exchange resin that retains acetyl-CoA but lets the AcCh pass [8, 9]. Other acetylated products, however, such as AcCar, pass through and give a spurious AcCh reading [7]. The liquid cation exchange assay utilizes a sodium tetraphenylboron (TPB), dissolved in an appropriate organic solvent, to stop the reaction. TPB reacts with charged quaternary ammonium compounds to form a complex that is extracted into the organic layer. The radiolabeled acetyl-CoA substrate is not extracted. The advantage of the liquid cation-exchange procedure is that most acetylated products other than AcCh are not basic enough to react with TPB [7, 10–12]. The presence of a carboxylic group on the AcCar molecule prevents the formation of a complex of AcCar with TPB at neutral pH. AcCar will form the complex optimally at pH 2.8 [13]. This selectivity gives the liquid cation-exchange procedure a reported advantage over the anion-exchange procedure [7]. The problems of AcCar contamination of product and the introduction of carnitine as a substrate are acute in investigating spermatozoa. Rat epididymal tissues, particularly the cauda, are very rich sources of carnitine [14].

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§ Abbreviations: AcCh, acetylcholine; ChAc, choline acetyltransferase; AcCar, acetylcarnitine; CarAc, carnitine acetyltransferase; TPB, tetraphenylboron; BETA, 2-benzoylethyltrimethylammonium chloride; NVP, 4-(1-naphthylvinyl)pyridine; *V*, velocity of enzymatic reaction; and *S*, substrate concentration.

The epididymal spermatozoa contain high levels of CarAc [15]; high CarAc levels can cause the formation of true AcCh [7].

The principal objectives of this investigation were: (1) to determine the extent to which sperm is capable of synthesizing AcCh, and (2) to determine whether classical or "true" ChAc is responsible for AcCh synthesis or if another enzyme (e.g. CarAc) is a primary contributor to AcCh synthesis.

METHODS

The areas investigated were: (1) a comparison of the anion-exchange resin method and the liquid cation-exchange method for the determination of putative AcCh synthesis in rat sperm, (2) a comparison of the choline substrate requirements and the kinetics of enzymatic synthesis of AcCh by neuronal tissue, rat sperm, and purified CarAc, and (3) a comparison of the effects of specific and nonspecific inhibitors of ChAc and CarAc in neuronal tissue, rat sperm, and purified CarAc, to clarify which enzyme is responsible for AcCh synthesis in rat sperm.

Rat sperm were obtained from the caudae of the epididymides of male Sprague–Dawley rats (375–475 g, Harlan Industries, Inc., Indianapolis, IN). The cauda from three rats were minced in Hanks' balanced salt solution, and then minced tissue was filtered through doubled 200 μm nylon mesh. The sperm were centrifuged at 3000 *g* for 10 min and resuspended in 10 ml of Hanks' balanced salt solution. This procedure was repeated four times to thoroughly wash the sperm. The final sperm suspension was sonically disrupted for two 30-sec intervals (Branson Sonifier 185; Branson Sonic Power Co., Danbury, CT). Aliquots of the sperm suspension were quick frozen in a dry ice/acetone bath and stored at −80°.

The brains were dissected from male ICR Swiss mice and homogenized in a 1:19 (w/v) Hanks' balanced salt solution. The homogenate was centrifuged at 10,000 *g* for 10 min. Aliquots of supernatant fluid were quick frozen in a dry ice/acetone bath and stored at −80°.

Purified CarAc from pigeon breast muscle was purchased from the Sigma Chemical Co. (St. Louis, MO).

Before the assay was started, 0.5% Triton X-100 (1 part) was added to the tissue homogenate (4 parts). The tissue homogenate was used in 0–50 μl quantities and was allowed to preincubate for at least 5 min at 37°. The reaction was started by adding 50 μl of [^{14}C]AcCoA substrate solution with the following components: 85 mM sodium phosphate buffer (pH 7.5), 367 mM NaCl, 0.65 mM EDTA, 0.075% bovine serum albumin, 0.50 mM [^{14}C]AcCoA (sp. act. = 3.56 mCi/mmole; Amersham, Arlington Heights, IL), and 0.17 mM physostigmine sulfate. The final incubation volume was 100 μl for all reactions. The final acetyl-CoA concentration was 0.25 mM for all reactions. Choline concentrations were varied from 0.05 to 1000 mM. Unless stated otherwise, reaction time was 30 min. Controls with no tissue or enzyme, were run at each choline concentration tested.

For the anion-exchange resin based assay the reactions were run in triplicate 12 × 75 mm test tubes at 37°. The reactions were stopped by adding a 0.50-ml aliquot of ice water. Three 0.50-ml ice water washes were passed over the anion-exchange resin AG 1-X8 (chloride form, 200–400 mesh; Bio-Rad Laboratories, Richmond, CA) in Pasteur pipettes. The washes were collected in liquid scintillation vials, 15 ml of ACS (Amersham) scintillant was added, and radioactivity was counted on a Packard Tri-Carb scintillation counter (Packard Instrument, Downers Grove, IL).

For the liquid cation-exchange procedure, the reactions were run in triplicate 1.5-ml Eppendorf micro-test tubes (Brinkmann Instruments, Westbury, NY) at 37°. The reactions were stopped by the addition of 250 μl butyronitrile (Eastman Organic Chemicals, Rochester, NY) in which 30 mg/ml sodium TPB (Sigma Chemical Co.) was dissolved. The reaction vials were vortexed, and then centrifuged at 2000 *g* for 10 min to separate the butyronitrile layer from the aqueous layer. A 125-μl aliquot from the top butyronitrile layer was counted for radioactivity in 10 ml of ACS. The enzyme inhibitors 4-(1-naphthylvinyl)pyridine (NVP; CalBiochem-Behring Corp., La Jolla, CA) (a specific inhibitor of ChAc [16–17]), 2-benzoylethyltrimethylammonium chloride (BETA) (an inhibitor of ChAc [18, 19]) and, as determined by this laboratory, CarAc, were tested. The enzyme inhibitors were added to the tissues before the reaction was initiated. NVP was shielded from light because of its photosensitivity. Controls had comparable enzyme inhibitor concentrations, but no tissue or enzyme. Protein was determined by the procedure of Lowry *et al.* [20].

RESULTS

A comparison between the anion-exchange resin based assay and the liquid cation-exchange based assay for acetylation activity in rat sperm revealed acetylated product formation independent of exogenous substrate in the former but not in the latter (Table 1). NVP (500 μM) did not appreciably alter this endogenous acetylase activity. BETA (500 μM) did abolish this endogenous acetylase activity. Addition of exogenous choline (8.25 mM) caused an increase in acetylation activity of 102.0 pmoles acetylated product·min^{-1}·(mg protein)$^{-1}$ in the anion-exchange labeled assay due to the formation of AcCh [188.5 pmoles total acetylated product·min^{-1}·(mg protein)$^{-1}$] compared to no substrate addition. An addition of 8.25 mM choline to the liquid cation-exchange assay gave an acetylation activity of 106.4 pmoles AcCh·min^{-1}·(mg protein)$^{-1}$. This compares well with the 102.0 pmoles AcCh·min^{-1}·(mg protein)$^{-1}$ observed in the anion-exchange assay. Use of carnitine as substrate (8.25 mM) resulted in a large increase of sperm acetylase activity to 15,663 pmoles AcCar·min^{-1}·(mg protein)$^{-1}$ with the anion-exchange resin procedure. This activity was not inhibited by NVP, but it was abolished by BETA (Table 1). Less than 4 per cent of this activity was detected by the liquid cation-exchange procedure. Given these data, the liquid cation-exchange procedure was superior to the anion-exchange procedure in screening out false

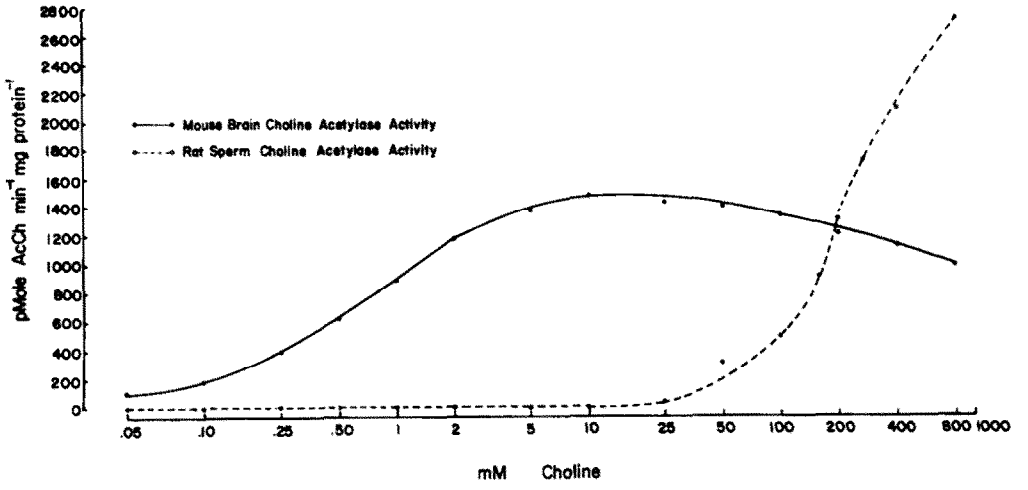

Fig. 1. Acetylcholine formation in mouse brain and rat sperm vs choline concentration. Tissue protein concentration: mouse brain = 0.533 mg protein/ml reaction mixture; rat sperm = 0.253 mg protein/ml reaction mixture.

Table 1. Comparison of rat sperm acetyltransferase activity using the anion-exchange resin method versus the liquid cation-exchange method*

				pmole AcPr·min^{-1}·(mg protein)$^{-1}$	
Choline	Carnitine	NVP	BETA	anion exchange resin	liquid cation exchange
—	—	—	—	86.5	1.8
—	—	0.5 mM	—	83.6	0.7
—	—	—	0.5 mM	0	0
8.25 mM	—	—	—	188.5	106.4
8.25 mM	—	0.5 mM	—	150.6	72.7
8.25 mM	—	—	0.5 mM	6.5	0
—	8.25 mM	—	—	15,663.0	602.7
—	8.25 mM	0.5 mM	—	16,004.0	661.4
—	8.25 mM	—	0.5 mM	10.9	0

* Incubation time = 15 min. AcPr = acetylated product. Tissue protein concentration = 0.884 mg protein/ml reaction mixture.

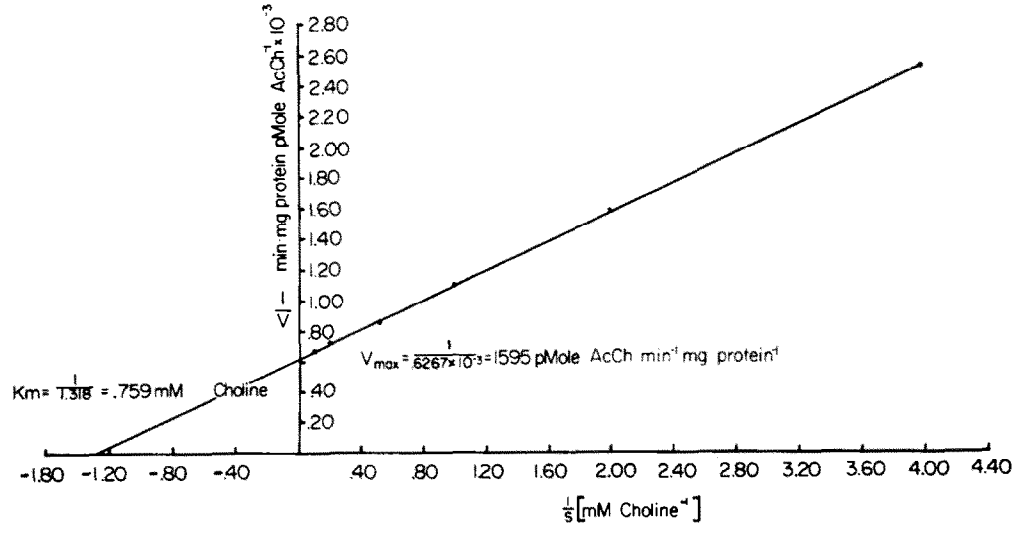

Fig. 2. Lineweaver–Burk plot of enzymatic formation of acetylcholine by mouse brain. Tissue protein concentration = 0.533 mg protein/ml reaction mixture.

Table 2. V_{max} and choline substrate K_m for enzymatic acetylcholine formation by mouse brain, rat sperm, and purified pigeon breast muscle carnitine acetyltransferase*

Tissue	$K_m \pm$ SD (mM choline)		$V_{max} \pm$ SD (pmole AcCh · min^{-1} · (mg protein)$^{-1}$)	
	Lineweaver–Burk†	Hanes‡	Lineweaver–Burk†	Hanes‡
Mouse brain	0.623 ± 0.151	0.600 ± 0.099	1603 ± 69	1620 ± 81
Rat sperm	207 ± 29	182 ± 40	3718 ± 108	3501 ± 187
Carnitine acetylase	50.7 ± 5.5	50.6 ± 7.8	(2.69 ± 0.17) × 10^5	(2.68 ± 0.78) × 10^5

* Each mouse brain value is the average of three different brain preparations. Each rat sperm value is the average of four determinations from two different sperm preparations. Each carnitine acetyltransferase value is the average of three determinations from the purified enzyme preparation.

† $\frac{1}{S}$ vs $\frac{1}{V}$.

‡ S/V vs S where S is the choline substrate concentration and V is the velocity of the reaction.

AcCh that was actually AcCar. Because of these data, the liquid cation-exchange procedure was utilized in all further assays to evaluate the formation of AcCh.

The next experiment was to determine which choline substrate concentrations would be conducive to enzymatic formation of AcCh and to compare rat sperm with mouse brain homogenate, a rich source of ChAc. Figure 1 illustrates nearly maximum saturation of ChAc by substrate for mouse brain at a range of choline concentrations (2–10 mM) where AcCh formation was minimal in rat sperm. AcCh formation in rat sperm was comparatively low until concentrations of choline were greater than 25 mM. In fact, the velocity of AcCh formation in rat sperm eventually overtook the maximum velocity observed in rat brain at a choline concentration greater than 250 mM. This is a strong indication that two different enzymes were involved, and that choline had a very low affinity for the enzyme responsible for choline acetylation in rat sperm. In the next experiment the V_{max} and K_m values for AcCh formation, using choline as substrate, were compared in mouse brain, rat sperm, and purified CarAc from pigeon breast muscle. The data are presented as double-reciprocal Lineweaver–Burk plots ($1/V$ vs $1/S$ where V velocity of the reaction and S is the substrate concentration) (Table 2 and Figs. 2–4) and Hanes plots (S/V vs S) (Table 2). The V_{max} of AcCh synthesis by rat sperm was approximately two times greater than by mouse brain (Table 2). The K_m with mouse brain compared favorably with rat brain ChAc (K_m = 0.770 mM choline [21]; K_m = 0.410 mM choline [22] and ox brain ChAc (K_m = 0.750 mM choline [23]. The K_m of choline was 80 times greater with purified carnitine acetylase and 300 times greater with rat sperm than

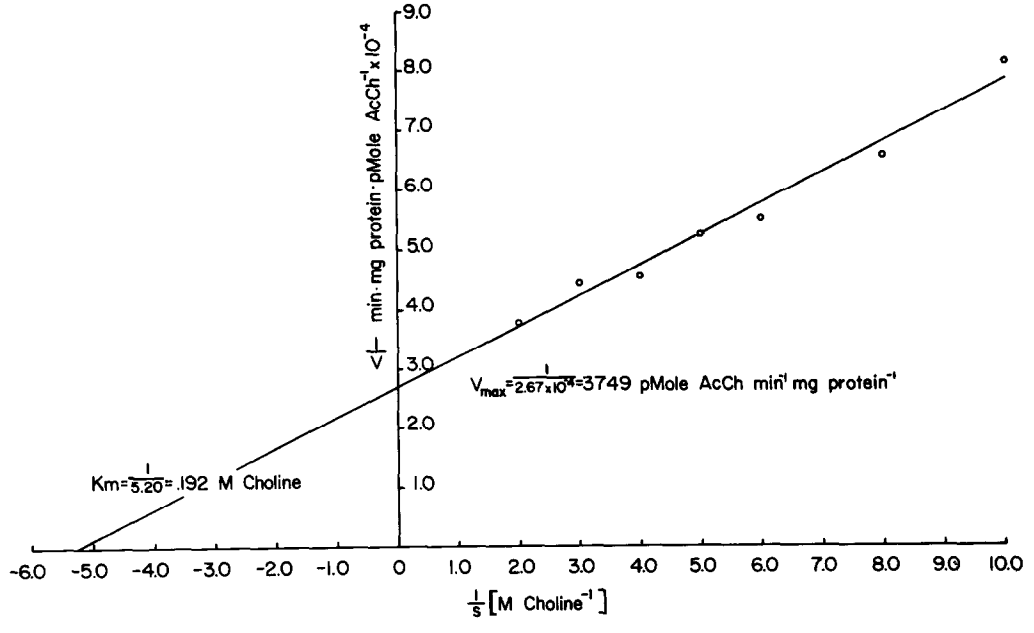

Fig. 3. Lineweaver–Burk plot of enzymatic formation of acetylcholine by rat sperm. Tissue protein concentration = 0.253 mg protein/ml reaction mixture.

Table 3. Effect of altering choline substrate concentrations with and without NVP and BETA on enzymatic formation of acetylcholine*

	Choline	NVP	BETA	pmole AcCh·min^{-1}·(mg protein)$^{-1}$	Relative activity†	%Inhibition‡
Mouse brain	2 mM	—	—	1432	1.00	—
	2 mM	0.5 mM	—	14	0.01	99
	2 mM	—	0.5 mM	0	0	100
	10 mM	—	—	1672	1.17	—
	10 mM	0.5 mM	—	33	0.02	98
	10 mM	—	0.5 mM	0	0	100
Rat sperm	2 mM	—	—	40	1.00	—
	2 mM	0.5 mM	—	19	0.48	52
	2 mM	—	0.5 mM	0	0	100
	10 mM	—	—	178	4.45	—
	10 mM	0.5 mM	—	67	1.68	62
	10 mM	—	0.5 mM	0	0	100
Carnitine acetyltransferase	2 mM	—	—	10,219	1.00	—
	2 mM	0.5 mM	—	9013	0.88	12
	2 mM	—	0.5 mM	208	0.02	98
	10 mM	—	—	42,742	4.18	—
	10 mM	0.5 mM	—	37,492	3.67	12
	10 mM	—	0.5 mM	560	0.05	99

* Tissue or enzyme protein concentrations: mouse brain = 0.770 mg protein/ml reaction mixture; rat sperm = 1.027 mg protein/ml reaction mixture; and carnitine acetyltransferase = 20.8 μg protein/ml reaction mixture.

† Relative activity where AcCh formation at 2 mM choline = 1.

‡ Per cent inhibition when compared to the control with no enzyme inhibitor present.

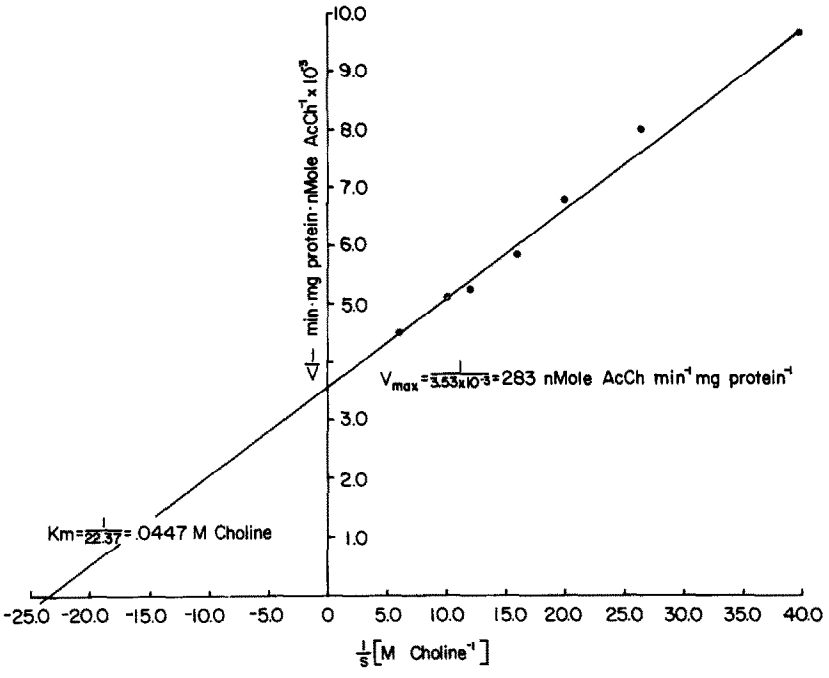

Fig. 4. Lineweaver–Burk plot of enzymatic formation of acetylcholine by purified pigeon breast muscle carnitine acetyltransferase. Enzyme protein concentration = 5.21 μg protein/ml reaction mixture.

with mouse brain (Table 2). This indicates that the lower affinity of choline substrate for the enzyme that synthesized AcCh was much lower in rat sperm than in mouse brain.

Detection of AcCh formation at concentrations of choline lower than those used in determining V_{max} and choline K_m has been observed for rat sperm and CarAc. It was not clear, with rat sperm, if this AcCh formation was catalyzed by true ChAc or by CarAc. The difference in AcCh formation between choline substrate concentrations of 2.0 and 10.0 mM was observed for mouse brain, rat sperm, and purified CarAc. If relatively low choline substrate concentrations are used relative to the K_m of choline with the enzyme forming the AcCh, then activity will be linearly proportional to substrate concentration. If this is true for the area of substrate concentration tested, then the ratio of activities with 10 mM choline and 2 mM choline will tend toward 5. If relatively high choline concentrations are used compared to the K_m, then there will be an approach to maximum velocity occurring near substrate saturation of the enzyme. If this is true for the area of substrate tested, then the ratio of activity with 10 mM choline to that with 2 mM choline will tend toward 1. Table 3 demonstrates that, for the choline substrate concentrations tested, mouse brain homogenate was near saturation (V 10 mM choline/V 2 mM choline = 1.17), rat sperm homogenate was near the linear portion of substrate dependence (V 10 mM choline/V 2 mM choline = 4.45), and AcCh formation by purified CarAc was near the linear portion of substrate dependence (V 10 mM choline/V 2 mM choline = 4.18). These data indicate that most of the AcCh formation in rat sperm with 2 and 10 mM choline was catalyzed by an enzyme whose K_m was much greater than the concentrations of choline used. Sperm utilizes choline as a poor substrate in a manner similar to that of purified pigeon breast muscle CarAc.

A specific inhibitor of ChAc was used to help clarify which enzymes were or were not responsible for AcCh product formation. NVP is a known inhibitor of ChAc and was tested to determine if classical or "true" ChAc was responsible for AcCh formation in sperm. NVP (500 μM) did not inhibit AcCar formation by rat sperm (Table 1) or by purified CarAc. BETA, an inhibitor of both ChAc and CarAc, was also tested. Table 3 shows that, with 2.0 and 10.0 mM choline, NVP (500 μM) essentially abolished ChAc activity in mouse brain. With 2.0 mM choline, NVP (500 μM) caused 52 per cent inhibition of AcCh formation in rat sperm and 12 per cent inhibition of AcCh formation by purified CarAc (Table 3). BETA (500 μM) essentially abolished all AcCh formation in the systems tested (Table 3).

DISCUSSION

The anion-exchange resin based assay gave an anomalously high value for AcCh synthesis in rat sperm compared to the liquid cation-exchange based assay (Table 1). The rat sperm demonstrated acetylation activity independent of exogenous substrate in the anion-exchange resin based assay, but not in the liquid cation-exchange based assay. Data in this investigation, reinforced by data in the scientific literature [7, 14, 15], strongly suggest that the acetylated product, synthesized independently of choline substrate, was AcCar. The evidence for this tentative conclusion is: (1) the endogenous acetylation activity independent of choline substrate was

not detected by the liquid cation-exchange procedure that is noted in the literature [7] and by data in this investigation (Table 1) to exclude the detection of AcCar, (2) rat sperm contain significant amounts of endogenous carnitine [14], and (3) data in the literature [15] and in this investigation (Table 1) demonstrate high CarAc in rat sperm. In previous investigations, the anion-exchange resin method was used to demonstrate putative ChAc activity and AcCh synthesis [4, 5]. The data in this investigation on acetylase activity in rat sperm independent of choline substrate correspond closely with putative ChAc reported in rat sperm by previous investigators [4]. Investigators have noted that putative ChAc activity and AcCh synthesis can actually be CarAc synthesizing AcCar. Rossier [7] has reviewed reports of AcCh synthesis in liver that were purported to be evidence of ChAc whereas it was actually AcCar synthesized by CarAc.

Choline substrate requirements for AcCh synthesis by mouse brain, rat sperm, and purified CarAc demonstrate that choline substrate affinity is much lower for purified CarAc and rat sperm than for mouse brain. The choline substrate K_m with rat sperm (207 mM choline) was approximately 300 times greater than with mouse brain (0.633 mM choline) and also 4 times greater than with purified CarAc from pigeon breast muscle (K_m = 50.7 mM choline). The V_{max} in rat sperm [3718 pmoles AcCh·min^{-1}·(mg protein)$^{-1}$] actually surpasses the V_{max} in mouse brain [1603 pmoles AcCh·min^{-1}·(mg protein)$^{-1}$]. The mouse brain choline substrate K_m is in very good agreement with other choline substrate neuronal K_m values [21–23]. These data indicate that AcCh synthesis in rat sperm is not catalyzed by classical or "true" ChAc as found in mouse brain and other neuronal tissue. Furthermore, the data in this investigation, demonstrating that purified CarAc can synthesize AcCh and that rat sperm can quite readily utilize carnitine to synthesize AcCar, strongly suggest that CarAc in rat sperm is responsible for AcCh synthesis. This conclusion is supported in scientific literature reporting that CarAc can synthesize AcCh [7] and that rat sperm are very high in CarAc activity [15]. Even at choline concentrations much lower than those concentrations used to determine K_m and V_{max} in sperm, there is evidence that an enzyme with a much higher K_m than "true" ChAc is responsible for AcCh synthesis (Table 3).

The implication of the choline substrate requirements for AcCh synthesis by purified CarAc is that high choline substrate concentrations may give rise to spurious acetylase activity that is due to CarAc activity. A probable example of this is an interesting paper that appeared recently reporting ChAc in the rat heart [24]. The investigators in the rat heart study used a choline substrate concentration of 30 mM. Considering that the K_m of choline substrate was 50.7 mM, as determined in this investigation for purified CarAc, the possibility must be raised that CarAc is responsible for the AcCh synthesized by rat heart. This possibility is reinforced by scientific literature reporting high levels of CarAc in the rat heart [25].

The issue of AcCh being synthesized by CarAc and the presence of true AcCh in the heart has been elegantly investigated by White and Wu [17] and Roskoski *et al.* [26]. White and Wu [17] demonstrated ChAc in rabbit heart by employing electrofocusing techniques to separate ChAc from CarAc. Roskoski *et al.* [26] concluded that ChAc was present in guinea pig heart through the use of bromoacetylcholine and AcCar as differential inhibitors of ChAc and CarAc.

NVP (500 μM), a well-known inhibitor of ChAc [16, 17], inhibits AcCh synthesis by mouse brain (99 per cent inhibition), rat sperm (52 per cent inhibition), and purified CarAc (12 per cent inhibition) to different degrees. These data further support the contention that another enzyme, rather than "true" ChAc, is at least partially responsible for AcCh synthesis in rat sperm. In this investigation, NVP did not inhibit the ability of rat sperm to synthesize AcCar (Table 1). In data not presented, NVP (500 μM) did not inhibit synthesis of AcCar by purified CarAc. These data indicate a partial substrate specificity for inhibition of a particular enzyme. BETA, a ChAc inhibitor [18, 19], has been shown to be a general inhibitor of ChAc and CarAc (Table 1). This suggests caution in utilizing only BETA as an inhibitor to determine putative ChAc activity.

It has been noted that ChAc from certain non-neuronal tissues may not be similar to neuronal ChAc and that there may exist specific and nonspecific ChAc with respect to choline or acetyl-CoA as substrate [27]. In rat sperm, however, the "nonspecific" ChAc appears to be CarAc. Purified CarAc is "nonspecific" in that it has a demonstrable ability to synthesize AcCh.

An interesting paper [6] reports ChAc activity in ram spermatozoa with a K_m of 0.02 mM choline and a V_{max} of 543 pmoles AcCh·min^{-1}·(mg protein)$^{-1}$. A species difference may be responsible for this difference in AcCh synthesis in ram sperm as opposed to rat sperm.

The data in this investigation demonstrate that rat sperm have the ability to synthesize AcCh at high enough choline substrate concentrations, but that the principal enzyme responsible for AcCh synthesis is not ChAc and is likely to be CarAc. If any "true" ChAc is present, it is in very minute amounts. Investigators and potential investigators in this area should note that, when examining for potential ChAc activity, rigorous methodology must be employed to distinguish between the AcCh synthesized by ChAc and AcCh synthesized by some other enzyme (e.g. CarAc).

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