A STUDY ON ACETYLCHOLINE ACTIVITY IN HUMAN PLACENTA AT DIFFERENT STAGES OF GESTATION PERIOD

by

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SUMMARY

Acetylcholine (ACH) activity in 83 placentas in I, II and III trimesters and post-term periods was estimated by "In Vitro" incubation of placental tissue from MTP (8-10 weeks), from MTP by hysterotomy or by intra-amniotic injection of methyl prostaglandins $PGF_2 \propto$ (14-20 weeks), from premature deliveries (28-34 weeks) from full term normals and twins deliveries (280 days) and from post-term deliveries (over 290 days).

The total ACH synthesis in ug/g of dry tissue was 39 (100%) in full-term placenta, 24 (62%) in MTP (8-10 weeks), 223 (572%) in hysterotomy, 90 (231%) in premature placentas, 52 (133%) in twins and 45 (115%) in post-term ones. Prostaglandins stimulated placental ACH activity considerably both "In Vivo" as well as "In vitro".

Introduction

Chand and Gaddum (1933) were the first to report the presence of acetylcholine (ACH) in human placental extracts. Hebb and Ratkovic (1962) observed the presence of choline-acetylase (cholineacetyltransferase—ChAcTr) activity; and Ruch *et al* (1976) confirmed the presence of cholinesterases (True-and Pseudo-ChE) in human placenta. Brahmayya (1968) made an "in vitro" comparative study of choline (Ch) and ACh fluxes in rats' brain and human placenta. Raghavan and Brahmayya (1970) described in detail the synthesis and release of ACh by "in vitro" incubated placental mince at term; and the relevant procedures and calculations. Brahmayya Sastry and Krishnamurty (1978) made a study, using a new technique, on the dynamics of ACh synthesis and release in "in vitro"—perfused single cotyledon of full-term human placenta.

Out of the sporadic reports on human placental ACh activity, mention must be

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made that Chang and Lim (1949) estimated ACh content in wet tissue of aborted placenta (88-103 ug/g) and compared it with that at term (37-49 ug/g). This present study was an attempt at systematic assessment of ACh activity at different stages of gestation period of human placenta (dry tissue).

Material and Methods

Within 2 minutes after placental delivery (full term, premature or post-mature vaginal or caesarean) the healthy human placenta was transferred for 30 minutes to a cold (5°C-10°C) and moist polythene bag kept in a refrigerator.

The placental tissue of the I trimester was obtained by operation for Medical Termination of Pregnancy (MTP) (a) of 8-12 weeks by suction evacuation and (b) of 16 weeks by hysterotomy (only one case). Pregnancy with reliable history of over 290 days was considered postmature.

Fine mince of random but fully representative parts of placental tissue was made in cold and a known weight of the mince was incubated "in vitro" at 37°C-38°C in glass tubes in an organ bath as described by Raghavan and Brahmayya Sastry (1970) except that phosphate-buffered and eserinised Ringer-Locke's (ERL) and oxygen were used. Incubation were made in triplicate. A 2-ml sample of the medium (supernatant) was drawn every 30 minutes from each tube in order to study the time course of ACh release. The incubation was terminated at the end of 2 hours by transferring the tubes to the refrigerator (5°-10°C).

The dry weight of the placental tissue was determined by drying up a known weight of the tissue in a hot air oven at 80°C for 3 to 5 days till its weight was reduced to three successive equal values. *The ACh was uniformly expressed as ug/g of dry weight of placental tissue.*

The extracts containing ACh were kept in cold at pH[4] till they were assayed on the arterial blood pressure of chloralosed and eviscerated cat as described by MacIntosh and Perry (1950). The free (liquid) and bound (tissue) fractions of ACh (FACh and BACh) were estimated and the total ACH synthesis and the ratio of FACh to BACH at the end of 2-hours incubation period were calculated.

For the reason that only one MTP (II trimester-16 weeks) by hysterotomy was available, MTP (16-20 weeks) was induced in about 18 hours each in 5 cases by intra-amniotic injection of methyl prostaglandins (PGF_2 10 ml of 250 ug/ml) giving about 8.5 ug/ml concentration in amniotic fluid).

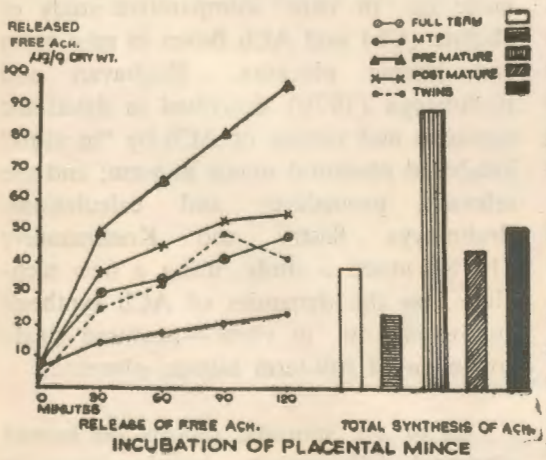

Fig. 1

Results

The different aspects of the results of 78 experiments are duly presented (Table I and chart) showing *especially* (1) the time course of release of FACh during incubation and (2) the total ACh synthesis, and the results of 5 $PGF_{2\alpha}$ experiments are properly compared in Table II.

TABLE I

Acetylcholine Activity (ug/g. Dry Weight) of "In Vitro" Incubated Placental Tissue

1	2		3	4		5		6
S. No.	Incubation		Ach Free (F) or Bound (B)	Healthy* Full term (40 weeks) (25 expts.)		Twins (38-40 weeks) (12 Expts)		P
	Period (min.)	Temp. (°C)		Mean** ±SE	%	Mean** ±SE	%	
1.	0	5-10	F	8.2 ±1.5	100	2.8 ±0.32	34	0.02
2.	0	5-10	B	37 ±2.3	100	38 ±2.5	103	0.7
3.	30	37-38	F	30.0 ±2.1	100	25 ±2.0	83	0.2
4.	60	37-38	F	35 ±2.4	100	33 ±2.5	94	0.7
5.	90	37-38	F	41 ±2.8	100	42 ±4.1	102	0.9
6.	120	37-38	F	49 ±3.1	100	50 ±4.5	102	0.7
7.	120	37-38	B	35 ±2.8	100	42 ±3.5	120	0.1
8.	120	37-38	F B	1.6 ±0.14	100	1.5 ±0.12	87	0.3
9.	0-120	37-38	Total (TS) synthesis	39 ±4.1	100	52 ±4.1	133	0.05
			Dry weight (%) ± SE	15.3 ±0.04	100	14.6 ±0.4	95	

1	6			7			8			9	
S. No.	MTP (8-10 weeks) (19 Expts)			Premature (28-34 weeks) (5 Expts)			Postmature (over 290 days) (16 Expts)			Hysterotomy (16 weeks) (1 Expt.)	
	Mean** ±SE	%	P	Mean** ±SE	%	P	Mean** ±SE	%	P	Value	%
1.	6.8 ±1.4	84	0.5	8.0 ±1.2	98	0.9	6.8 ±1.8	83	0.8	32	391
2.	29 ±6.0	78	0.2	60.0 ±2.8	162	<0.001	45 ±4.6	122	0.7	70	189
3.	15 ±1.9	50	<0.001	50 ±4.5	167	<0.001	36 ±2.7	120	0.6	131	437
4.	18.0 ±2.0	51	<0.001	66 ±5.8	189	<0.001	45 ±3.4	129	0.4	171	489
5.	21 ±2.4	51	<0.001	82 ±7.2	200	<0.001	53 ±3.9	129	0.6	215	524
6.	24 ±2.5	49	<0.001	97 ±8.0	198	<0.001	56 ±5.2	114	0.8	247	504
7.	25 ±3.4	71	<0.05	61 ±1.7	174	<0.001	45 ±4.5	129	0.7	78	223
8.	1.2 ±0.2	75	0.02	1.6 ±0.13	100	0.9	1.5 ±0.27	94	0.1	3.2	200
9.	24 ±3.2	62	0.01	90 ±7.6	231	<0.001	45 ±6.3	115	0.2	223	572
	13.2 ±0.4	86		14.2 ±0.5	93		15.7 ±0.7	103		13.1	86

* Values (vaginal and caesarean deliveries at full term).
** Mean of averages of triplicates of each related experiment.
P = Level of significance of difference between the mean of the group and that of the corresponding full term value.

TABLE II

*Acetylcholine Activity (ug/g. Dry Wt.) of 'In Vitro' Incubated Placental Tissue Comparison among (A) Normal Healthy Full-term Placenta (25) (B) Placental Tissue of MTP (G) by Hysterotomy at 16 Weeks (1) and (B) by prostaglandings * at 14-20 Weeks (5)*

1	2		3	4		5		6		
S. No.	Incubation		Ach Free (F) Bound (B)	Healthy Full-term (25 Expts.)		Hysterotomy (16 weeks) (1 Expt)		$PGF_2\alpha$-Injected* (5 Expts.)		
	Period (min.)	Temp. (°C)		Mean ± SE	%	Mean	%	± SE	%	P
1.	0	5-10	F	8.2 ±1.5	100	32	391	140 ±58	1707	<0.001
2.	0	5-10	B	37 ±2.3	100	70	189	158 ±65	427	<0.001
6.	120	37-38	F	49 ±3.1	100	247	504	611 200	1247	<0.001
7.	120	37-38	B	35 ±2.8	100	78	223	132 ±52	377	<0.001
8.	120	37-38	F/B	1.6 ±0.14	100	3.2	200	4.6 ±1.18	288	<0.001
9.	0-120	37-38	Total synthesis (TS)	39 ±4.1	100	223	572	445 ±238	1141	<0.001
10.			Dry weight (%) ± SE	15.3 ±0.04	100	13.1	86	12.7 ±0.63	83	<0.001

* Dose by intra-amniotic injection of $PGF_2\alpha$ 10 ml (250 ug/ml).

Note: Expulsion of placenta occurred about 18 hours after injection of $PGF_2\alpha$.

P: Denotes the level of significance of difference between means of this group and full-term values

The normal full-term delivered (280 days—25 cases—Table I column 4) contained vaginal (15) and caesarean (10) which showed no statistically significant variations (P value 0.2 to 0.8) in all the parameters of study of ACh activity.

The time course of release of FACh into the incubate shows that the rate and extent of release was lowest in MTP and highest in premature delivery; the rest being in between and fairly close to each other (Chart and Table I, column 6 and 7 and 4, 5 and 8). Compared to normal full-term placental ACh values: (a) the total ACh synthesis during 2 hour incubation shows statistically significant lower values (24/39 or 62%) in MTP and higher ones (90/39 or 231%) in premature delivery (28-34 weeks) and the rest of the parameters of study also are similar (Chart and Table I column 4, 6, 7) (b) *the free/bound ratio* at the end of 2 hours of incubation was lower (75%) and statistically significant only in MTP; and is comparable in the rest. At 16 weeks the ACh values are higher (column 9). The FACh liberation from bound ACh reserves in placental tissue of the early I trimester seems smaller than in the rest. (c) *$PGF_{2\alpha}$ induced abortion and hysterotomy (II trimester—Table II)*: At zero-incubation itself for 2 hours, the free and bound ACh values were respectively higher (391% and 189%) in placental tissue of hysterotomy (column 5) and far higher in $PGF_{2\alpha}$-aborted one (col. 6—1707% and 427%) compared with normals (col. 4). This comparision suggests that the placental tissue ACh could have been raised in *'in vivo' by* $PGF_2 \propto$ (8.5 ug/ml) incubation in the womb itself. In *"in vitro"* incubation for 2 hours at 37°C-38°C the Free/Bound ratio was 200% in hysterotomy placenta and 228% in $PGF_{2\alpha}$ -aborted placenta. The ACh synthesis respectively was 572% and 1141%; showing that $PGF_2 \propto$ *"in vitro"* incubation increased further the ACh turnover zero incubation. The higher values in 5 cases of $PGF_2 \propto$-induced abortion are all statistically significant (col. 6 and 4). Comparison of placental ACh values of hysterotomy (col. 5) with $PGF_2 \propto$-induced abortion (col. 6) suggests that II trimester placental values are likely to be naturally high compared to normal (col. 4); regardless of $PGF_2 \propto$ action.

The dry weights of placenta were 86% (MTP), 86% (Hysterotomy) and 83% ($PGF_2 \propto$) compared to III trimester and post-mature dry weight values, suggesting that placental tissue of I and II trimester have higher water content than the more mature ones (Table I and II).

Discussion

It has been established that all the 3 essential components of the ACh system (Ch, ChAcTr and ChE) for synthesis and hydrolysis of ACh, are present in plenty in human placenta, a nerve-free organ (see introduction).

In evolution, the role of ACh in non-nervous tissue precedes that in nervous tissue; and plays important role in limb maturation, growth and regeneration in salamanders (Vernandikis, 1973). Harbison *et al* (1976) attributes to ACh the function of regulating foetal growth and development. Tuchmann-Duplessis (1972) correlated the activity of cholinergic system with the development of syncitiotrophoblast during the first 6 months of gestation. Harbison *et al* (1976) reported that peak concentration of ChAcTr and ChE occur in placenta at about 22 weeks of gestation but declines thereafter.

The II trimester $PGF\alpha$ -MTP ACh values were in support of those of the single MTP by hysterotomy (see "Results" and

Table II). In view of this the ACh values along the gestation period are listed in sequence (see summary) which show peak ACh value in II trimester. Thus the results of this systematic study are not only in line with earlier sporadic reports, some of which are mentioned earlier, but also lend support to the view that there is functional correlation between growth and development of womb structures and placental ACh activity at various stages of gestation. Ach may be playing a vital role as donor of methyl groups in this most important period.

Ach values given in relation to dry weight of placenta threw light on the water content of placenta which was found higher in I and II trimester (Table I).

It is well known that Ach stimulates Prostaglandin release from chromaffin cells of adrenal gland (Ramnell *et al* 1966). Incidentally it is seen from the present study that $PGF_{2\alpha}$ incubation *"in vivo"* (8.5 ug/ml) in amniotic fluid and *"in vitro"* at 38°C for 2 hours enormously accelerated Ach activity to 1707% and 1141% respectively (Table II col. 5 and 6). Thus Ach and Prostaglandins seems to have mutually complementary action on placenta. These results support the views that Ach plays a role in determining the duration of pregnancy and in including uterine contractions.

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