ACETYLCHOLINE ACTIVITY IN HUMAN PLACENTA IN MATERNAL DIABETES MELLITUS AND INFECTIONS

by

M. SATYANARAYANA, K. R. RAJESHWARI,
G. RAMKRISHNAM RAJU AND P. BRAHMAYYA SASTRY

SUMMARY

A study was made on the effects of maternal diabetes mellitus and of infections on placental acetylcholine (ACh) activity. A 2-hour "In Vitro" incubation (in triplicate) of placental mince in maternal diabetes mellitus was made and its Ach activity compared with that in normal, healthy and full term placenta. The mean Ach synthesis (ug./g. of dry tissue) in diabetes was 129% of the normal mean Ach synthesis value which was 60 ug./g. ± SE 2.3. In contrast, in maternal infections like infectious hepatitis with jaundice it was 61% and in pulmonary tuberculosis 63%.

Satyanarayana and Brahmayya Sastry (1982) reported reduction (74%) in placental true cholinesterase (AChE) in maternal diabetes mellitus which situation might have lead to the increased Ach activity (129%) in diabetes mellitus in this study. The toxins of maternal infections might have reduced the Ach activity (61%-63%).

Introduction

Since the first report in 1933 by Chang and Gaddum that *human placenta,* a nerve-free organ, contained acetaylcholine (ACh), several scientists including Brahmayya Sastry and his associates showed that: (1) there is much more ACh in human placenta than in nervous system, (2) synthesis, storage and destruction of the ubiquitous ACh depended on a system (ACh system) comprising 4 components: (a) choline (Ch) that provides the building material, (b) the enzyme cholineacetyltransferase (ChACTr) which promotes ACh synthesis (c) ultramicroscopic vesicles that store large part of ACh (d) the enzyme cholinesterase (AChE) which breaks down (hydrolyses) ACh to Ch and acetic acid; and that these components are all present as much in human placenta as in nervous system.

While there is well-documented evidence that ACh, in tiny amounts, plays part in nerve impulse transmission, there are only postulations, although with accumulating evidence, that ACh is associated with the placental trophoblast and that it plays its role in pregnancy, probably by donating its methyl groups, for vascularisation and

From the Departments of Physiology and of Obstetrics and Gynaecology, Andhra Medical College, Visakhapatnam-530 002 (A.P.).

growth of uterus, early growth and regulation of foetal organs, development of foetal nervous system and determining the duration of pregnancy and onset of labour. It is interesting to know from Vernandikis (1973) that, in evolution, ACh plays important role in growth, development and regulation of non-nervous organs preceding the development of nervous system. Brahmayya Sastry and his associates by their periodical but systematic work from 1958 to 1983 determine placental ACh activity (1) as conditioned by physiological factors, (2) along gestation period and (3) in abnormal states of pregnancy like maternal diseases, foeto-placental abnormalities and delivery complications. There are very few reports in literature on ACh in abnormal states of pregnancy.

In this paper the results of study on influence of maternal diabetes mellitus on placental ACh activity are given. Their contrast with those in maternal infections (a) infective hepatitis with jaundice and (b) active pulmonary tuberculosis is also shown.

Materials and Methods

This study was made on 8 selected cases of maternal diabetes mellitus (6 vaginal and 2 caesarean-delivered); and on maternal infectious diseases (infective hepatitis with jaundice (5) and active pulmonary tuberculosis (1) in vaginal deliveries, *as diseases of contrast*; and all of them compared with 33 cases of healthy, full term deliveries (22 vaginal and 11 caesarean deliveries). Standard methods were used for evaluation of healthy and diseased patients prior to onset of labour or in labour. Relevant data was collected from the labour ward or labour room records.

In diabetes mellitus the random blood sugar was 125 mg% to 190 mg%, urine sugar +++ and in 2 cases there was ketosis. In infectious hepatitis with jaundice the serum bilirubin was between 8 mg% to 10 mg% and urinary bile salts were +++. In pulmonary tuberculosis the lesion was active and evident. *All the cases studied were multipara and delivered live babies except one in infectious hepatitis.*

Within 2 minutes following placental delivery, the placenta was transferred to a cold polythene bag moistened with Ringer-Locke's fluid and left in a refrigerator (about 4°C) for 30 minutes. Random but fully representative blocks of placental tissue were cut from the foetal to the maternal surface and fine mince of the same was made in cold Ringer-Locke's fluid. A known weight of the mince was incubated "*In vitro*" at 37°C in wide glass tubes in an organ bath and all ACh values were estimated as described by Brahmayya and Sastry (1962) and Raghavan and Brahmayya Sastry (1970) except that phosphate-buffered and eserinised Ringer-Locke's (ERL) fluid and oxygen were used. These two modifications did not affect ACh activity (Brahmayya Sastry and Krishnal activity (Brahmayya Sastry and Krishnamurty, 1978). Incubation was made, in triplicate, and for a 2-*hour period.* The non-incubated mince in control tubes were left in the refrigerator for a corresponding period. A 2 ml sample of the incubated medium (supernatant) was drawn every half-hour for study of the time-course of release of free ACh. The incubation was terminated at the end of 2 hours by transferring the tubes to the refrigerator (4°C).

The free ACh (FAch), the Bound ACh (BAch), FAch/BAch ratio at 2 hours and the total ACh synthesis (TS) *during incubation were all estimated.* The dry weight of the placental tissue was determined by exposing known weights of placental tissue

to 80°C in a hot air oven, usually for 3-5 days and determining the decreasing dry weight every 12 hours till 2 consecutive values became equal. The different parameters of ACh activity are uniformly expressed as ug/g of dry weight of placental tissue.

Results

The results given in the two tables are clear and self explanatory not only with reference to the experimental data but also the experimental design. Expression of ACh values against dry weight of placental tissue eliminates the effects of variation of water content of placenta on the data on different parameters of ACh activity.

In Table I, the FACh and BACh along the time course of incubation at 4°C and at 37°C; the F/B ratio at the end of 2-hour incubation, and the TS during the incubation period do not show any statistically significant difference between vaginally-delivered (22) and caesarean-delivered (11) placentas (col. 3 and 4 and "P" 0.2 to 0.9). Hence, the data of all the normal and full-term placentas (33) are complied

TABLE I

Acetylcholine (ACh) Activity (μg./g. dry wet.) of Incubated Tissue of Healthy and Full-term Human Placenta-comparison of Vaginal- and caesarean-delivered Placentas (33 *Experiments*)

1		2	3	4		5
Incubation Period (min)	Temp. (°C)	ACh Free (F) Bound (B) Total synthesis (TS)	Vaginal (22) Mean ±SE	Caesarean (11) Mean ±SE	'p' value	Vaginal & Caesarean (33) Mean ±SE
0	4	F	6.0 ± 1.2	4.0 ± 0.8	0.2	5.4 ± 0.9
0	4	B	47.0 ± 2.3	40.0 ± 3.4	0.2	44.0 ± 2.0
30	37	F	34.0 ± 2.7	33.0 ± 2.9	0.6	34.0 ± 2.1
60	37	F	44.0 ± 2.9	43.0 ± 2.8	0.9	43.0 ± 2.1
90	37	F	53.0 ± 3.1	52.0 ± 3.1	0.9	53.0 ± 2.3
120	37	F	62.0 ± 3.4	62.0 ± 3.0	0.9	62.0 ± 2.5
120	37	B	49.0 ± 2.8	43.0 ± 3.5	0.2	47.0 ± 2.2
120	37	F/B	1.3 ± 0.1	1.6 ± 0.1	0.3	1.4 ± 0.05
120	37	TS	59.0 ± 3.3	61.0 ± 2.5	0.5	60.0 ± 2.3

Col. 5: "p" values do not show statistically significant variations between data of col. 3 & 4.
Col. 6: Therefore gives data computed from the original values of col. 3 & 4. *This data is taken as normal for all related comparisons in health and disease.*

and *analysed from the basic values (col. 5); and thus the baseline data, which is essential and important for comparison with that in abnormal states is provided.* The FACh is higher than BACh (F/B 1.4) and increases steeply and steadily along the time course of incubation at 37°C (col. 5).

In Table II, effects are shown on placental ACh activity of maternal diabetes mellitus (8), infectious hepatitis (5) and active pulmonary tuberculosis (though one case). This data is compared with normal, full term data (33) of Tab. I and Col. 5 and expressed as per cent of normal. *In diabetes* (col. 3) all the crucial values are increased uniformly (115% to 129%) and significantly ("p" 0.05 to 0.01); in *hepatitis* (col. 4) all the values are decreased markedly (55% to 64%) and significantly ("p" 0.01 to 0.001); and *in tuberculosis* also they are considerably reduced (55%-81%). However, the *steepness in increase in FACh* during incubation at 37°C remained normal in the three conditions. The *extent of release of Ach* (F/B) is normal in diabetes but is much less in hepatitis and tuberculosis. The TS is increased in diabetes (129%) but considerably reduced in hepatitis (60%) and in tuberculosis (63%).

The mean dry weight *per cents* ± SE of 22 normal vaginal-delivered and 11 caesarean-delivered placentas were 14.7 ± 0.4 and 15.8 ± 1.0 respectively; and those in diabetes (8), in hepatitis (5) and in tuberculosis (1) were 14.7 ± 0.3, 15.4 ± 0.6 and 13.3 respectively. The range of variation in dry weight (13.3% to 15.8%) justifies the objective of dry weight estimation.

Discussion

This paper presents data on changes in ACh activity in maternal diabetes mellitus, a metabolic, endocrinal cum immunologic disorder (Fox and McPath, 1969); as against infectious hepatitis and pulmonary tuberculosis both of which are infectious diseases. Jones *et al* (1976) reported that, by and large, only a few placentas in diabetes showed changes within normal limits while the rest of them exhibited changes described by Fox and McPath (1969) which, briefly stated, are: (a) maturation disturbances and (b) antigen-antibody reactions due to abnormal *insulin-binding* to the sites of pathology.

The EMG studies of Martin and Spicer (1973) showed that ACh and the enzyme AChE that hydrolyses ACh are localised in placental syncitiotrophoblast. Enzymes are quite sensitive to altered cell environment and cell function; and hence, in these studies AChE showed reduction in certain natal disorders while the pseudo-ChE which is, though high and probably associated with the bulky connective tissue, remained more stable.

A look at Tab. II col. 3 shows that, in diabetes, BACh even at 4°C-incubation, FACh and BACh and especially the more important TS at 37°C-incubation are significantly increased to 115% to 129% compared to normals (Tab. I col. 5); while in hepatitis the same parameters of ACh were decreased to 55% to 64% and in tuberculosis to 75% to 63% (Tab. II col. 4 and 5). Of all the parameters of ACh activity studied, *TS is the one which fully reflects the overall ACh changes at 37°C and it is incrseased to* 129% *in diabetes, to* 60% *in hepatitis with statistical significance and* to 63% in tuberculosis (1 case).

In diabetes mellitus, (Satyanarayana and Brahmayya Sastry, 1982) the more sensitive AChE is reduced to 74% of the normal and this could be the cause for increase (TS 129%) in ACh activity here. That AChE is decreased and ACh increased in

TABLE II

Acetylcholine (ACh) Activity in Incubated Tissue of Human Placenta in Diabetes Mellitus (8), *Infective Hepatitis with Jaundice* (5) *and in Pulmonary Tuberculosis* (1): *compared with normals* (33 *of Table I col.* 5)

1		2	3			4			5	
Incubation Period (min)	Temp. (°C)	Ach Free (F) Bound (B) Total synth. (TS)	Diabetes mellitus Mean ±SE	% of Normal	P value	Infective hepatitis Mean ±SE	% of Normal	P value	Pulmonary Tuberculosis Value	% of normal
0	4	F	2.7 ± 0.3	(50)	0.2	1.1 ± 0.1	(20)	0.05	1.4	(26)
0	4	B	55.0 ± 4.3	(125)	0.02	27.0 ± 2.2	(61)	0.01	33.0	(75)
30	37	F	39.0 ± 3.2	(115)	0.3	19.0 ± 1.3	(56)	0.01	20.0	(59)
60	37	F	51.0 ± 4.4	(119)	0.1	24.0 ± 1.5	(56)	0.001	26.0	(61)
90	37	F	64.0 ± 5.5	(121)	0.02	29.0 ± 1.6	(55)	0.001	29.0	(55)
120	37	F	76.0 ± 6.7	(123)	0.02	36.0 ± 2.1	(58)	0.001	35.0	(57)
120	37	B	58.0 ± 5.1	(123)	0.05	30.0 ± 2.4	(64)	0.01	38.0	(81)
120	37	F/B	1.3 ± 0.14	(93)	0.8	1.2 ± 0.1	(86)	0.3	0.93	(66)
120	37	TS	77.0 ± 7.0	(129)	0.01	36.0 ± 2.3	(60)	0.001	38.0	(63)

1. Diabetes mellitus (col. 3) FACh at 90 min. & 120 min., BACh and TS are increased significantly (121%—129%).
2. Infective hepatitis (col. 4). There is uniform and significant decrease (55% to 64%) in FACh, BACh and TS.
3. Pulmonary tuberculosis (col. 5). There is uniform decrease (55%-81% in FCh, BACh and TS.

Note the contrast between 1 *and* 2 *and* 3 *in the data.*

another immunological disorder, Rh-incompatibility, reported by Satyanarayana and Brahmayya Sastry (1985), lends support to the conclusion regarding diabetes. Similarly, the finding of Artner and Bauman (1977) that maternal levels of human placental lactogen (HPL) and human chorionic gonadotropin (HCG) are increased in diabetes mellitus is also another reassuring finding similar to ours.

In the infectious diseases in question, considerable reduction in ACh activity may be due to circulating toxins in maternal blood. Support is also lent to our observations in this paper by the report by Lindberg and Nilsson (1973) that HPL decreased in abnormal pregnancies like eclampsia, retarded foetal growth, perinatal foetal mortality etc., except in diabetes mellitus and Rh-isoimmunisation.

Acknowledgements

The authors are beholden to the Government of Andhra Pradesh, the Director of Medical Education, the Principal, Andhra Medical College and the Professor of Physiology for the liberal facilities given for this research. They are thankful to Dr. Sashiprabha and her associates at the labour ward and labour room as well as at the operation theatre for their keen interest and co-operation. They are also grateful to the Rockefeller Foundation, New York and U.G.C., New Delhi, for their gift equipment some of which was used in this research.

References

1. Artner, J. and Bauman, G. H.: Wien. Klin. Wochenschr. **89/15**: 524, 1977 (summary in English).
2. Brahmayya Sastry, P.: Acetylcholine in perfused human placenta. Ind. J. Physiol. Pharmacol. **6**: 2, 15, 1962.
3. Brahmayya Sastry, P. and Krishnamurty, A.: Ind. J. Med. Res., **68**: 867, 1978.
4. Fox, H. and McPath: Pathology of the placenta in maternal diabetes mellitus. Obstet. Gynec., **34**: 792, 1969.
5. Jones, C. J. P., Fox, H. and McPath: Placental changes in gestational diabetes—an ultrastructural study. Obstet. Gynec., **48**: 274, 1976.
6. Lindberg, B. S. and Nilsson, B. A.: J. Obstet. Gynec. Brit. C'wealth., **80**: 1046, 1973.
7. Martin, B. J. and Spicer, S. S.: Anat. Rec., **175**: 15, 1973.
8. Raghavan, K. S. and Brahmayya Sastry, P.: Ind. J. Med. Res., **58**: 1712, 1970.
9. Satyanarayana, M. and Brahmayya Sastry, P.: Biomedicine, **2**: 33, 1982.
10. Satyanarayana, M. and Brahmayya Sastry, P.: J. Obstet. Gynec. India, **35**: 1065, 1985.
11. Vernandikis, A.: Progr. Brain Res., **40**: 231, 1973.