Effect of Taurine and Hypotaurine on Mouse Embryo Development

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Summary

To investigate the effect of taurine and hypotaurine on the in vitro development of mouse embryos from 1-cell to blastocyst, the embryos were incubated in four media, i.e., M16, M16 plus EDTA, M16 plus taurine and M16 plus hypotaurine.The incorporation of methionine and the incorporation and oxidation of glucose were also compared in embryos cultured in these media. Both of number of embryos developed to four-cell stage and that to blastocyst were much larger in incubation in M16 plus taurine medium than in M16, although the numbers in the former medium were less than those in M16 plus EDTA. No significant difference in the incorporation of ^{3}H-methionine was found between the embryos incubated in M16 plus taurine, M16 and M16 plus hypotaurine media throughout the period of development examined. On the other hand, the incorporation and oxidation rates of ^{14}C-glucose were significantly higher ($p<0.05$) in the embryos cultured in M16 plus taurine compared with those in M16 at the period of 40-45 h after hCG injection . These results indicate that taurine, but not hypotaurine, has a beneficial effect on the embryo development in mice, perhaps through the ability to overcome the 2-cell block in the species.

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Key words : taurine, hypotaurine, two-cell block, mouse embryo, methionine.

Introduction

In vitro development of mouse embryos from one cell to blastocyst can occur only in a limited number of inbred strains and F_1-hybrids. Embryos of many other strains and random bred colonies can undergo from the first cleavage to the two-cell stage, but usually fail to develop further thereafter[1-3]. The cause or mechanism of this phenomenon, the so-called two-cell block, has not been clarified yet.

Taurine and its precursor, hypotaurine, are sulfur-containing β-amino acids, and it has been revealed that they present at relatively high concentration in female reproductive tract fluids in several mammals[4-6] and in semen[7] and sperm acrosomes[8] in human.

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Much has been known about the role of taurine playing in the processes of sperm capacitation and in vitro fertilization (IVF) in various species of mammals. Hamster spermatozoa require the presence of taurine or hypotaurine in the culture medium to maintain their motility and viability during in vitro capacitation[9]. Also, the fertilizing capacity of hamster spermatozoa in IVF is enhanced by the addition of taurine and hypotaurine to the medium[10-11]. In the cow, the presence of hypotaurine in the medium also stimulates the in vitro fertilization[12]. However, although human spermatozoa are capacitated faster in the medium with taurine added[13-14], their fertilizing ability in vitro seems not to be increased by the treatment[15], and mouse spermatozoa do not need the addition of taurine to exhibit their full fertilizing ability[16].

Compared with these findings, the role of taurine in the mammalian embryo development is less clear. The present study, therefore, was conducted to examine the effect of taurine and hypotaurine on the in vitro embryo development during the early preimplantation stages in mice, including their effects on the incorporation of methionine and the incorporation and oxidation of glucose of embryos at these stages.

Materials and Methods

ICR strain mice of 5-8 weeks old were used. They were kept under controlled temperature (20±1°C) and lighting (12 h dark and 12 h light) conditions and allowed free access to commercial solid food and water. Female animals were injected ip. with 5 IU pregnant mare serum gonadotrophin (PMSG : Peamex, Sankyo-zoki K.K.) followed with 5 IU human chorionic gonadotrophin (hCG : Puberogen, Sankyo-zoki K.K.) 48 h later for the induction of superovulation. After hCG injection the females were placed with fertile males and checked for the presence of copulation plugs on the following morning. One-, two-, four- and eight-cell embryos and morulae and/or blastocysts were collected from animals which had vaginal plugs by breaking oviducts or by flushing uteri 20, 30, 40, 45, 50, 60, 90 and 95 h after hCG injection, respectively.

Experiment 1 : The experiment was designed to examine the effect of taurine and hypotaurine on the in vitro development of preimplantation mouse embryos. The basic culture medium was Whittingham's M16[17]. Fertilized ova or one-cell embryos were collected 30 h after hCG injection. Embryos were washed three times in the same medium and transferred (10 embryos each) into a drop (50μl) of one of the following media ; M16 medium, M16 medium plus 5.0 mM taurine, M16 medium plus 5.0 mM hypotaurine and M16 medium plus 50mM EDTA. The drops were covered with paraffin oil and cultured for 70 h at 37°C and in 5 % CO_2 in air. Developmentof embryos were assessed with phase contrast microscope at 23 and 70 h of the culture.

Experiment 2 : Embryos of one-, two-, four- and eight-cell and morula and blastocyst stages were collected at 20, 30, 40, 45, 50, 60, 90 and 95 h after hCG injection, respectively.

The embryos showing normal morphology at each stage were selected, and ten each of them were put into a small plastic test tube (diameter 1.0cm, depth 3.0cm) containing 100μl of M16 medium, M16 medium plus 5.0 mM taurine or M16 medium plus 5.0 mM hypotaurine and ^{3}H-methionine (1.85×10^{-4}Bq/tube, specific activity : 1 mCi/ml). After the medium was covered with paraffin oil, the tubes were incubated for 1 h at 37°C under 5 % CO_2 in air. The incubation was finished by addition of cold TCA to a final concentration of 5 %. Acid insoluble materials were separated by millipore filtration (scwp 8μ) and washed with 5 % TCA and ethyl alcohol several times.

Experiment 3 : Embryos of one-, two-, four- and eight-cell and morula and blastocyst stages were collected as in Experiment 2. The rates of incorporation and oxidation of ^{14}C-glucose were measured according to Brinster[18]. Ten embryos at each stage were transferred to a small test tube containing M16 medium, M16 medium plus 5.0 mM taurine or M16 medium plus 5.0 mM hypotaurine and ^{14}C-glucose (1.85×10^{-4}Bq : Specific activity : 3.5 mCi/ml). One ml Hyamine was taken into another test tube and two tubes were put into a scintillation vial. The vial were then air-tightened and incubated for 3 h at 37 °C under 5 % CO_2 in air. At the end of incubation, cold PCA was added to a final concentration of 5 %. Acid insoluble materials were separated and washed with PCA as descrived above.

Radio activities of the acid insoluble materials (^{3}H and ^{14}C) and hyamine (1ml) were determined by a scintillation counter (TRI-CARB, Packard) by the use of scintillation cocktail (toluene : 1000ml, POPOP : 300mg, PPO : 4g) and 8 samples were used for each observation.

The data obtained were analyzed statistically by Fischer's exact probability test in Experiment 1 and Student's t test in Experiments 2 and 3.

Results

Experiment 1 :

The results of Experiment 1 are shown in Table 1. Taurine had the ability to overcome the two-cell block occurring in the in vitro culture and promote the embryo development to blastocysts in this species. However, this effect of taurine did not reach that of EDTA, and then significant differences in the rates of embryo development to the four-cell and blastocyst stages were observed between in M16 plus EDTA and in M16 plus taurine and between in M16 plus taurine and in M16. Addition of hypotaurine to M16 had no beneficial effect on the mouse embryo development.

Experiment 2 :

As shown in Fig. 1, the incorporation of ^{3}H-methionine was lower in embryos collected at 45 h after hCG injection compared with those at any other time after hCG

Table 1. The effect of taurine and hypotaurine on the development of 1-cell mouse embryos.

Medium additives	n	No. embryos developing (%)		
		2cell	4cell	blastocyst
None	120	102 (85)	2 (2)[a]	0 (0)[a]
EDTA	121	108 (89)	98 (81)[b]	79 (65)[b]
taurine 5.0mM	119	100 (84)	40 (34)[c]	26 (22)[c]
hypotaurine 5.0mM	120	108 (90)	10 (8)[a]	4 (3)[a]

[abc]Means with different superscripts differ from each other ($p<0.05$) ; Fisher's exact probability test.

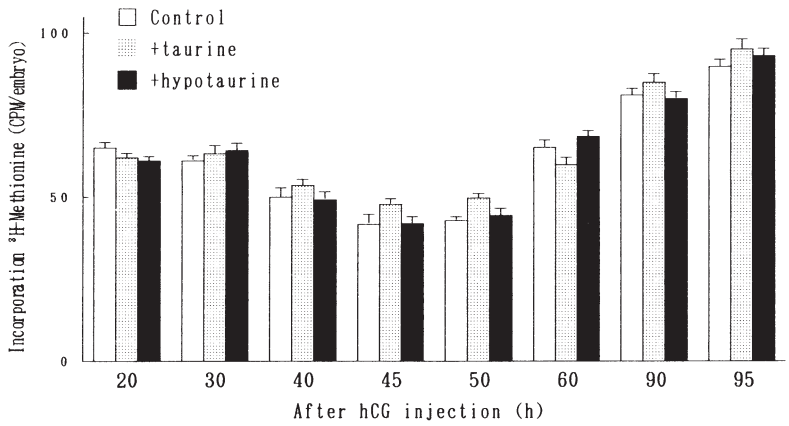

Fig 1. The effect of taurine and hypotaurine on incorporation of ^{3}H-methionine into the early mouse embryos.
(M±SEM, n=8)

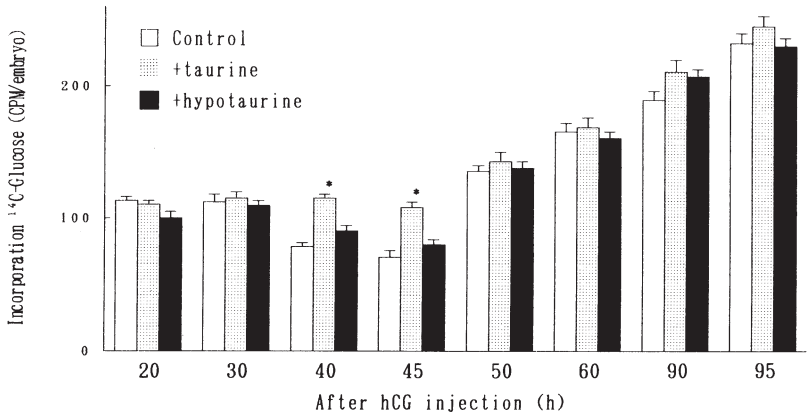

Fig 2. The effect of taurine and hypotaurine on incorporation of ^{14}C-glucose into the early mouse embryos.
(M±SEM, n=8, *$P<0.05$: compared within the same time.)

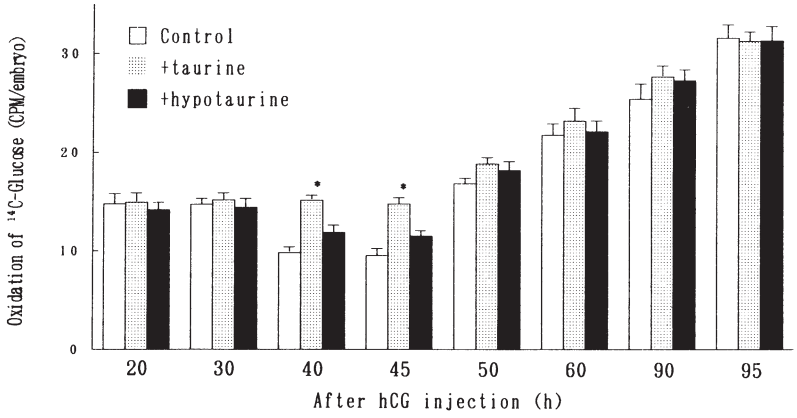

Fig 3. The effect of taurine and hypotaurine on oxidation of ^{14}C-glucose into the early mouse embryos.
(M±SEM, n=8, *P<0.05 : compared within the same time.)

injection in all groups cultured in different media. The incorporation started to increase from 50 h after hCG injection and continued to rise up to the period of 95 h in all groups. No significant differences were found in the incorporation rate of methionine between M16 plus taurine, M16 plus hypotaurine and M16 media throughout the whole stages of embryo development.

Experiment 3 :

Both the incorporation and oxidation of ^{14}C-glucose were significantly higher in M16 plus taurine than in M16 and M16 plus hypotaurine medium at 40 and 45 h after hCG injection ($P<0.05$) (Fig.2 and 3). In M16 plus hypotaurine medium, the incorporation and oxidation of ^{14}C-glucose were almost similar to those in M16 medium. The incorporation and oxidation of glucose increased gradually from 50 h to 95 h after hCG injection as the age of embryos cultured progressed, but no significant effect of taurine and hypotaurine on the glucose metabolism was observed throughout these stages.

Discussion

In our culture experiment, the rate of mouse embryo development from one-cell to four-cell stages was higher in the medium with taurine (34 %) than in the control medium (2 %) but lower than that in the medium with EDTA (81 %). These results indicate that taurine has an effect to make mouse embryos overcome the two-cell block in the in vitro culture, although the effect is less potent than that of EDTA. Miller and Schultz[6] reported that taurine concentration of rabbit uterine fluid was 3.41 mM. In the preliminary experiments, 0.1, 1.0, 5.0 and 10 mM taurine were added to the culture medium and good promotion of the embryo development was obtained in the culture with 1.0 and 5.

0 mM, a little better result with 5.0 mM. Dumoulin et al.[19] presented evidence that taurine may promote the development of mouse embryos by protecting them from the adverse effect of high K^+ concentrations in female reproductive tract fluids. In the mouse, the K^+ concentration in oviduct fluid was found to vary from 18 mM to 30 mM, depending on the stages of the estrous cycle and in the segment of the oviduct studied[20-21]. Taurine and K^+ are known to interact in the sperm capacitation and fertilization processes[22]. The high concentrations of both taurine and K^+ in reproductive tract fluids suggests that taurine also protects spermatozoa from the effect of high K^+ concentration by suppressing the excessive rise in the $Na^+ -K^+ -$ATPase activity[23]. Usually, mammalian sperm capacitation and fertilization take place in uterine and oviductal fluids and the fluids also make up the environment in which the embryo develops during the preimplantation stages. It is possible to consider, therefore, that taurine affects the embryonic development in the same manner as it influences sperm motility, capacitation and fertilization, i.e. through the protection of embryos against the high K^+ concentrations in female reproductive tract fluids.

High concentration of taurine in female reproductive tract fluid and in embryos of rabbit was reported by Li and Foot[24]. They stated that taurine might serve as antioxidants. Antioxidants such as catalase, superoxide dismutase (SOD) and taurine are considered to be beneficial as additives to synthetic media, because they can serve as scavengers of toxic free radicals in media lacking serum, serum albumin, or other macromolecules as in more complex media[25]. Nonogaki et al.[26] reported that antioxidants might serve as scavengers of toxic free radicals in media for overcoming two-cell block in mice. In addition to this function, taurine also may exert its effect as a chelating agent. The benefical effects of chelating transitional metals on the embryo development in the in vitro culture have been discussed in detail recently[27-29].

We did not find any significant effect of hypotaurine on mouse embryo development in the present study. This may be due to the lack of hypotaurine dehydrogenase which convert hypotaurine to taurine. Our findings contradict with those of Dumoulin et al.[30] who used random-bred (CD-1 and NMRI) mouse, while we used ICR mouse. So the difference in the strain of mice used may be a cause of the inconsistency.

When considering all the results of incorporation experiments together, we suppose that the cell block in the in vitro embryo development occured within a relatively narrow range of time, during 40-45 h after hCG injection, at least in ICR mouse. This result is supported by our previous work that almost the same result was obtained by addition of EDTA[31]. Causes of the cell block have been intensively investigated during the past decade, but no clear interpretation has been obtained yet. In general, the phenomenon is regarded to appear coinciding with the time of embryonic genome activation which occurs at a particular stage of embryo development characteristic to respective species[32], and, therefore, the failure of this activation is the most plausible as the cause

of cell block. However, this explanation seems to be too simple. First, it is very inconceivable that the genome activation takes place suddenly and massively at a very restricted stage of embryo development. Instead, it is more probable that the activation occurs more gradually and progressively over the longer period of preimplantation development, albeit very little transcriptional activity can be detected at the very early stage of embryo development, e.g., after one or a few cleavages have occurred. In other words, estimation of the timing of genome activation depends on the sensitivity of methods employed and there may be embryonic genome activity as early as during the first cell cycle[33].

The second of oppositions comes from the fact that the occurrence of cell block coincides with the transition of eggs from oviductal to the uterine stages in several species. The concept that the demands of the embryos change during the progress along the female reproductive tract has been amply supported by the studies showing profound changes in their morphology and ultrastructure, as well as the shift of amino acid transport mechanism and the change in the responsiveness to growth factors along with the embryo development[34]. Changes in the morphology of mitochondria between the oviductal and uterine embryos[35] may be particularly significant in view of the evidence for the disturbed metabolism in cultured embryos.

There are alternative explanations for the occurrence of cell block in the in vitro development. One possibility is the problem of inadequate energy production. In most species, judicious adjustment of nutrient composition of culture media or addition of suitable energy source to the media can overcome the block and allows a substantial proportion of embryos to develop up to the blastocyst stage even in vitro. This means that understanding metabolic pathways of embryos and/or their substrate and nutrient preferences well can lead to striking advances in our ability to support embryo development in vitro. Nevertheless, under the culture conditions presently available, the metabolism of cultured embryos is still not the same as that in vivo.[36]. Another feasible explanation for the cell block is the production of toxic superoxides and free oxygen radicals which may impair the cell in a variety of ways, such as by perturbing the function of cell membrances, by elevating the intracellular pH and/or by disturbing the mitochondrial function.

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マウス胚発生に及ぼすタウリン，ヒポタウリンの影響

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要 約

ICR 系マウス初期胚に及ぼすタウリン，ヒポタウリン添加の影響を調べるため，M16 液，M16 液＋EDTA， M16 液＋タウリン，M16 液＋ヒポタウリン中で1-cell 胚を培養して4-cell， blastocyst への発生率を調べた。タウリン添加区においては，対照区と比較して，EDTA 添加区ほどではないが4-cell, 胚盤胞への発生率が高まった。ヒポタウリン添加によっては発生率の増加はみられなかった。さらに初期胚のメチオニン，グルコースの取り込みにタウリン，ヒポタウリン添加が及ぼす影響を調べるため，hCG 投与後20，30，40，45,50，60，90，95時間の卵子を採取し， M16液，M16 液＋タウリン，M16 液＋ヒポタウリン中で^{3}H-メチオニン，^{14}C-グルコースを取り込ませた。メチオニンの取り込みにおいては有意差はみられなかったが，グルコースの取り込みにおいては hCG 投与後40，45時間において，タウリン添加区で対照区と比較して，取り込みが有意に高まった。これらのことから，マウス胚体外培養で起こる2-cell block に対し，タウリンが何か抑制的な影響をもつことが示唆された。

キーワード：タウリン，ヒポタウリン，2-cell block，マウス胚，メチオニン