

## VII. Summary

### **The influence of different incubation temperatures and hypoxia on the cAMP content in heart muscle cells from chicken embryos (*Gallus gallus f. domestica*)**

Basal cAMP concentration in heart muscle cells and its response to isoproterenol was determined on D18 and D20 in chicken embryos (*Gallus gallus f. domestica*). In addition, some morphological parameters were monitored. Two series of experiments were conducted, in which incubation temperatures were altered throughout the incubation period and one in which oxygen was reduced in incubation air. In the temperature experiment the control group was continuously incubated at 37,5°C. After D15, incubation temperature was decreased to 35,0°C in the cold group and was increased to 38,5°C in the warm group. In the second experiment, the control group was continuously incubated in atmosphere containing 21 % oxygen. From D12 on, the hypoxia-group was incubated under mildly reduced oxygen (15% O<sub>2</sub>). Eggs were taken from all groups on D18 and D20, respectively. The hearts were prepared and digested with Trypsin in order to form a cell suspension. cAMP content was determined in the cells by means of cAMP enzyme immunoassay (BIOTRAK). In addition, the weight of the embryos and the hearts were determined.

The embryo mass show an age dependant increase of 45% from D18 to D20. This corresponds with the values found by FREEMAN und VINCE (1974). A decrease in the incubation temperature led to a developmental delay with smaller increases in weight. An increase in temperature induced a larger increase in embryo mass. According to BURGGREN (1998), every organism displays a temperature dependent development rate. The results of this study confirm this thesis with regard to embryo mass. Decreasing oxygen to 15% in incubation air had no effects on embryo mass.

Heart mass increased 25% under normal incubation conditions (37,5°, 21% O<sub>2</sub>) from D18 to the D20. A decrease in the incubation temperature of 35,0°C led to an increase of 50% within this period. When incubation temperature was increased to 38,5°C, an increase in the heart mass was no longer detectable.

A decrease in the oxygen content of the incubation air to 15% led to 12% reduction in heart mass as compared to the control group (21% O<sub>2</sub>). No differences were detectable on D20.

Previous studies (McLEAN et al., 1975; ORELLANA und BROWN, 1985; SMITH und PAPPANO, 1985; EPSTEIN et al., 1987) show that basal cAMP-concentration in the

embryonal heart muscle cells of the house chicken decrease until D16 and then remains on a plateau until hatching. These findings were confirmed in the present study. No significant differences could be detected in the experimental groups from D18 to D20. cAMP-levels in all groups remained around 1-3 amol/cell.

ISO, a selective  $\beta$ -agonist, stimulates the  $\beta$ -adrenoreceptor-g-protein-adenylate-system, thus increasing cellular cAMP-concentration. The extent of this stimulation decreased with the age of the embryos. Under standard conditions (37,5°C) cAMP increase after stimulation with ISO sank from 250% on day 18 on 75% on day 20. After decreasing incubation temperature to 35,0°C, the age dependant decrease in the responsiveness of the cellular cAMP-concentration to ISO could no longer be detected. After increasing incubation temperature to 38,5°C the age dependant decrease in the responsiveness of the cellular cAMP-concentration by ISO remained. However, at 38,5°C the values were somewhat smaller than in the control group (37,5°C).

A long-term treatment of embryonic heart cells with noradrenaline leads to a decrease in the responsiveness of the cAMP-concentration through ISO in vitro (REITHMANN et al., 1987). Under normal incubation conditions, the catecholamine-concentration in plasma of chicken embryos increased towards the end of incubation period (BLUMROEDER und TÖNHARDT, 2002).

When incubation temperature was reduced, this rise in catecholamine concentration was suppressed, while an increase in temperature had no distinct effect.

The increasing noradrenaline concentration in the blood of the chicken embryos towards the end of incubation period considered as a cause for the decrease of the sympathetic responsiveness of the cellular cAMP-content.

A decrease in the oxygen content of the incubation air led to a responsiveness of the cellular cAMP-content through ISO on D18, in contrast to the situation observed in the control group (21% O<sub>2</sub>), where ISO responsiveness did not occur until D20. From D18 to D20, no further decrease could be observed.

The results of the present study contribute to a characterization of the regulation and feedback of the cardial performance shortly before hatch.