MITIGATION OF CAFFEINE-INDUCED FETOPATHY
IN MICE BY PRETREATMENT WITH β-ADRENERGIC
BLOCKING AGENTS*

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Abstract—In a previous experiment, fetopathic effects of caffeine were significantly reduced by pretreatment with propranolol at dosage levels of 2.5 to 10 mg/kg. The present experiments were undertaken to investigate the relation between time intervals of propranolol pretreatment and its effect on reducing fetopathy. Furthermore, the effect of timolol, another β-adrenergic blocking agent, on reducing fetopathy was compared with that of propranolol. Propranolol (5 mg/kg) administered 15, 30 or 60 minutes before caffeine treatment significantly reduced the caffeine-induced fetopathy. The optimal effect was found when propranolol was given 30 minutes before caffeine. The reduction in fetopathy by timolol pretreatment was comparable to that of propranolol. The results lend support to the hypothesis that the fetopathic effect of caffeine is linked with released catecholamines in maternal or fetal tissues of mice.

Fetopathic effects of large doses of caffeine or other methyl xanthines have been demonstrated in mice (1, 2, 3) and rats (4, 5). Also, a high concentration of caffeine in the culture medium was reported to be effective as a mutagen and a synergizer of mutagenic activity (6). However, whether these two activities of the compound are associated with each other or are independent, is yet unknown. Although no human malformation has been attributed to caffeine and little teratogenic (7) or mutagenic (6) danger to man has been suggested, it is desirable to elucidate the mechanism of the fetopathic action, as there may be other chemical substances which involve the same mechanism.

In a previous experiment, we demonstrated that fetopathic effects of caffeine were significantly reduced by pretreatment with the β-adrenergic blocking agent propranolol at dosage levels of 2.5 to 10 mg/kg (8). In consideration of the established pharmacological action of caffeine, we hypothesized that some β-adrenergic actions of catecholamines, which are released by caffeine (9) in maternal or fetal tissues, cause fetal circulatory disturbances and that the disturbance leads to fetal abnormalities.

To provide verification for the hypothesis, mitigation of caffeine-induced fetopathy by pretreatment with β-adrenergic blocking agents should be established as a first step. Therefore, the present experiments were planned to investigate the relationship between time intervals of propranolol pretreatment and its effect on reducing fetopathy caused by caffeine with the idea that there must be an optimal blocking time interval if the action of released

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Catecholamines is blocked by a $\beta$-adrenergic blocker in the reduction of the fetopathy. In addition, the reducing effect of propranolol was compared with that of timolol (Fig. 1), another $\beta$-adrenergic blocking agent (10, 11).

**MATERIALS AND METHODS**

ICR-JCL mice were purchased from Japan CLEA Co. at 4 weeks of age and raised for approximately 6 weeks at the Nippon Merck-Banyu Laboratories. During this period as well as throughout the experiment the animals were kept in an air-conditioned room at $22\pm1^\circ\text{C}$ and $55\pm5\%$ humidity and fed a stock diet (CA-1, Japan CLEA Co.) with fresh tap water provided ad libitum. Nulliparous females of about 10 weeks of age were mated overnight (17 hr) with adult males of the same strain. Copulation was established the next morning by the presence of a vaginal plug and the day designated as day 1 of gestation. The presumably pregnant females were divided into 5 experimental and 2 control groups for the first series of experiments and 2 experimental and one control groups for the second series of experiments. All the pregnant animals were treated on day 13 of gestation in the following manner.

Experimental animals in the first series (Table 1) were administered 5 mg/kg propranolol (Inderal injection, Sumitomo Chemical Co., Ltd.) i.v. through the tail vein 0, 5, 15, 30 or 60 min prior to i.p. injection of 200 mg/kg caffeine dissolved in saline. The groups of these animals were designated as P-C, P5-C, P15-C, P30-C and P60-C, respectively. Blocking action of propranolol when administered after caffeine treatment was not investigated since fetopathic or teratogenic alterations are generally regarded as being irreversible. Control animals were given an i.v. injection of either saline or 200 mg/kg caffeine. These control groups were designated as S-S and S-C.

In the second series of experiments, the effects of timolol on reducing caffeine-induced fetopathy were studied and compared with those of propranolol. Animals in the experimental groups were given an i.v. injection of either 5 mg/kg propranolol or timolol dissolved...
in saline followed 30 min later by an i.p. injection of 225 mg/kg of caffeine. These groups were designated as P-C' and T-C'. Control animals were treated i.v. with saline followed immediately by an i.p. injection of 225 mg/kg caffeine. These control groups were designated as S-S and S-C'. All the caffeine injections were given at 10 a.m.

Pregnant females were sacrificed on day 19 of gestation. The number of implantation sites and signs of death such as fetal resorption or maceration were checked in situ. The live fetuses were examined for external abnormalities and cleft palate. The data was statistically analyzed by the Wilcoxon's Rank Sum Test.

RESULTS

The results obtained from the first series of experiments are summarized in Table 1. The frequency of resorptions and abnormal fetuses in the S-S group was comparable to our background control data in the same strain of mice, which have been accumulated over the past 10 years. In the S-C group, the incidences of abnormal fetuses and pregnant females with abnormal fetuses, as expected, was significantly increased compared to the S-S group. In the experimental groups, the pretreatment with 5 mg/kg propranolol at given time intervals reduced the rates of abnormal fetuses and pregnant females with abnormal fetuses to varying extents; in the P15-C, P30-C and P60-C groups the rates of both parameters were significantly reduced compared to the S-C group. The optimal time for reducing fetopathy was obtained when propranolol was administered 30 min prior to the caffeine injection. The abnormality most frequently observed was cleft palate (Table 2). The incidences of cleft palate in the P15-C, P30-C and P60-C groups were significantly lower than that of the S-C group. Other types of abnormalities observed in control and experimental groups with statistically insignificant incidences were brachygnathia, subcutaneous hematoma, digital defects, open eyelid, encephaly, umbilical hernia and grossopalatine fusion.

The results of the second series of experiments are summarized in Table 3. Both propranolol and timolol administered i.v. at 5 mg/kg significantly reduced the frequencies of caffeine-induced abnormal fetuses as compared with the frequency in the S-C' group. The incidence of pregnant females with abnormal fetuses was significantly increased in the S-C' group and decreased by pretreatment with propranolol or timolol, though without significant differences. These abnormalities are classified in Table 4. Types of abnormalities were similar to those observed in the previous series of experiments. Both propranolol and timolol significantly reduced the frequencies of fetuses with cleft palate, brachygnathia and subcutaneous hematoma. However, incidences of cleft palate were less in cases of timolol, but the difference was not statistically significant.

DISCUSSION

In these series of experiments the fetopathic effect of caffeine in mice was significantly reduced by maternal pretreatment with the β-adrenergic blocking agents, propranolol and timolol. The optimal time for reducing fetopathy was when propranolol was administered 30 min before caffeine treatment. The β-adrenergic blocking action of propranolol
### Table 1. Reduction of fetopathic effects of caffeine by pretreatment with propranolol at various time intervals

| Group  | Pretreatment\(^1\) (i.v.) | Treatment\(^1\) (i.p.) | Time interval (min) | No. of litters | Total implants | Resorption % | Abnormal fetuses % | Females with abnormal fetuses % |
|--------|---------------------------|------------------------|---------------------|----------------|---------------|--------------|-------------------|-------------------------------|
| Control |                          |                        |                     |                |               |              |                   |                               |
| S-S    | saline                    | saline                 | 0                   | 18             | 225           | 8.0          | 0.5               | 5.6                           |
| S-C    | saline                    | caffeine               | 200 mg/kg           | 0              | 268           | 8.6          | 21.2\(^2\)         | 85.0\(^2\)                   |
| P-C    | propranolol               | caffeine               | 200 mg/kg           | 0              | 286           | 10.1         | 17.9              | 76.2                          |
| P5-C   | propranolol               | caffeine               | 200 mg/kg           | 5              | 256           | 9.0          | 16.7              | 85.0                          |
| P15-C  | propranolol               | caffeine               | 200 mg/kg           | 15             | 239           | 5.0          | 10.6\(^3\)        | 44.4\(^4\)                   |
| P30-C  | propranolol               | caffeine               | 200 mg/kg           | 30             | 269           | 9.3          | 6.6\(^5\)         | 40.0\(^6\)                   |
| P60-C  | propranolol               | caffeine               | 200 mg/kg           | 60             | 258           | 8.1          | 10.1\(^7\)        | 50.0\(^8\)                   |

\(^1\) Volume of the solutions pretreated or treated was 5 or 10 ml/kg respectively.
\(^2\) Significant at P < 0.01 compared with S-S (the Wilcoxon’s Rank Sum Test).
\(^3\) Significant at P < 0.01 compared with S-C (the Wilcoxon’s Rank Sum Test).
\(^4\) Significant at P < 0.05 compared with S-C (the Wilcoxon’s Rank Sum Test).

### Table 2. Reduction of caffeine-induced abnormalities by pretreatment with propranolol at various time intervals

| Group   | Live fetuses | cleft palate % | brachygnathia % | subcutaneous hematoma % | other malformations % |
|---------|--------------|----------------|-----------------|-------------------------|-----------------------|
| Control |              |                |                 |                         |                       |
| S-S     | 209          | 0.5            | 0               | 0                       | 0                     |
| S-C     | 245          | 20.8\(^1\)     | 0.8             | 2.4                     | 1.2                   |
| P-C     | 257          | 17.5           | 0.4             | 1.6                     | 1.2                   |
| P5-C    | 233          | 15.9           | 0.9             | 1.3                     | 1.3                   |
| P15-C   | 227          | 10.1\(^2\)     | 0               | 0.9                     | 0.4                   |
| P30-C   | 244          | 6.6\(^2\)      | 0               | 0                       | 0                     |
| P60-C   | 237          | 7.2\(^2\)      | 0               | 0.8                     | 2.5                   |

\(^1\) Significant at P < 0.01 compared with S-S (the Wilcoxon’s Rank Sum Test).
\(^2\) Significant at P < 0.01 compared with S-C (the Wilcoxon’s Rank Sum Test).
### Table 3. Reduction of fetopathic effects of caffeine by pretreatment with propranolol or timolol at 30 min intervals

| Group | Pretreatment<sup>1</sup> (i.v.) | Treatment<sup>1</sup> (i.p.) | No. of litters | Total implants | Resorption | Abnormal fetuses<sup>2</sup> | Females with abnormal fetuses<sup>2</sup> |
|-------|--------------------------------|-----------------------------|----------------|----------------|------------|----------------|-------------------------------|
| Control | S-S               | saline                     | 18             | 225            | 8.0        | 0.5            | 5.6                           |
|        | S-C'             | saline                     | 21             | 274            | 12.8       | 38.1<sup>2</sup> | 81.0<sup>2</sup>              |
| Experimental | P-C'         | propranolol 5 mg/kg        | 21             | 294            | 8.5        | 15.2<sup>4</sup> | 61.9                          |
|        | T-C'             | timolol 5 mg/kg            | 21             | 261            | 9.6        | 10.2<sup>2</sup> | 57.1                          |

<sup>1</sup> Volume of the solutions pretreated or treated was 5 or 10 ml/kg respectively.

<sup>2</sup> Significant at P<0.01 compared with S-S (the Wilcoxon's Rank Sum Test).

<sup>3</sup> Significant at P<0.01 compared with S-C' (the Wilcoxon's Rank Sum Test).

<sup>4</sup> Significant at P<0.05 compared with S-C' (the Wilcoxon's Rank Sum Test).

### Table 4. Reduction of caffeine-induced abnormalities by pretreatment with propranolol or timolol at 30 min intervals

| Group | Live fetuses | Cleft palate | Brachygnathia | Subcutaneous hematoma | Other malformations |
|-------|--------------|--------------|---------------|-----------------------|---------------------|
|       |              | %            | %             | %                     | %                   |
| Control | S-S       | 209          | 0.5           | 0                     | 0                   |
|        | S-C'      | 239          | 36.4<sup>1</sup> | 5.9<sup>1</sup> | 14.6<sup>1</sup> | 3.3<sup>2</sup> |
| Experimental | P-C' | 269          | 12.3<sup>3</sup> | 0<sup>3</sup> | 1.5<sup>2</sup> | 3.3 |
|        | T-C'      | 236          | 10.2<sup>3</sup> | 0.8<sup>3</sup> | 1.7<sup>2</sup> | 1.3 |

<sup>1</sup> Significant at P<0.01 compared with S-S (the Wilcoxon's Rank Sum Test).

<sup>2</sup> Significant at P<0.05 compared with S-S (the Wilcoxon's Rank Sum Test).

<sup>3</sup> Significant at P<0.01 compared with S-C' (the Wilcoxon's Rank Sum Test).
in the mouse fetus is assumed to be long lasting, as this blocking action reportedly lasts 10 hr in the ovine fetus (12). Therefore, the 30 min time interval required for the reduction in fetopathy may be due to a difference in drug distribution rate or placental transfer rate between the two compounds, if the β-blocking action is to be significantly elicited in the fetus to the extent that fetopathy will be reduced. The reduction in fetopathy by timolol pretreatment was comparable to that of propranolol, although the β-adrenergic blocking action of the former is several times more potent than the latter (9, 10, 13). These results lend support to the hypothesis that the teratogenic or fetopathic effect of caffeine is linked with the release of catecholamines in maternal or fetal tissues of mice.

The possibility that caffeine directly affects genetic materials should still be considered, as even adenine and its normal derivatives, which have a chemical structure similar to caffeine induce malformations in mice and rats (14, 15, 16, 17, 18). Additional related experiments are in progress in this laboratory.

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