Laboratory Investigations

Decreased hypothalamic prostaglandin D_2 and prostaglandin E_2 contents during isoflurane anaesthesia in rats

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This study was undertaken to evaluate the effect of isoflurane anaesthesia on the hypothalamic contents of both prostaglandin D_2 and E_2 which affect the sleep-wakefulness cycle. Sixty-three Wistar rats were divided into three equal groups, control, isoflurane and recovery groups. Twenty-one rats of the control did not receive isoflurane. In the other groups 21 rats received isoflurane 2% for 30 min and 21 received isoflurane 2% for 30 min and were allowed to recover their usual behaviours, including righting reflex, spontaneously. The hypothalamus was removed and the contents of PGD_2 and PGE_2 were measured by enzyme immunoassay. The PGD_2 content in the hypothalamus was 397.9 ± 226.0 $pg \cdot g^{-1}$ for the control group, 134.2 ± 41.2 $pg \cdot g^{-1}$ for the isoflurane group and 269.1 ± 124.6 $pg \cdot g^{-1}$ for the recovery group, respectively. The hypothalamic PGE_2 contents were 381.4 ± 139.0 $pg \cdot g^{-1}$ for the control group, 183.3 ± 26.4 $pg \cdot g^{-1}$ for the isoflurane group and 312.2 ± 96.0 $pg \cdot g^{-1}$ for the recovery group, respectively. The hypothalamic PGD_2 and PGE_2 contents in the isoflurane group were lower ($P < 0.05$) than those in the control and recovery groups, while both the PGD_2 and PGE_2 contents of the control and the recovery groups were similar. We conclude that decreased hypothalamic PGD_2 and PGE_2 contents may be related to some manifestations of general anaesthesia with isoflurane.

Cette étude vise à évaluer l'effet de l'isoflurane sur la teneur en prostaglandines D_2 et E_2 de l'hypothalamus, substances qui affectent le cycle du sommeil et de l'éveil. Soixante-six rats Wistar sont repartis en trois groupes identiques: contrôle, isoflurane et récupération. Vingt-et-un rats du groupe contrôle ne reçoivent pas d'isoflurane. Dans les deux autres groupes, 21 rats reçoivent de l'isoflurane 2% pendant 30 min et 21 rats reçoivent de l'isoflurane pendant 30 min suivi de la récupération spontanée de leur comportement usuel incluant le réflexe de redressement. L'hypothalamus est alors isolé et le contenu en PGD_2 et PGE_2 est mesuré par épreuve immunologique. La teneur de l'hypothalamus en PGD_2 est respectivement 397,9 ± 226,0 $pg \cdot g^{-1}$ dans le group contrôle, 134,2 ± 41,2 $pg \cdot g^{-1}$ dans le groupe isoflurane et de 269,1 ± 124,6 $pg \cdot g^{-1}$ dans le groupe récupération. Le contenu hypothalamique de PGE_2 est respectivement de 381,4 ± 139,0 $pg \cdot g^{-1}$ pour le groupe contrôle, 183,3 ± 26,4 $pg \cdot g^{-1}$ pour le groupe isoflurane, et de 312,2 ± 96,0 $pg \cdot g^{-1}$ pour le groupe récupération. La teneur de hypothalamus en PGD_2 et en PGE_2 du groupe isoflurane est plus basse ($P < 0,05$) que celle du groupe contrôle et du groupe récupération. Les auteurs concluent que la diminution du contenu hypothalamique de PGD_2 et de PGE_2 pourrait être un effet de l'anesthésie à l'isoflurane.

Key words
ANAESTHESIA: depth;
ANAESTHETICS, VOLATILE: isoflurane;
HORMONES: prostaglandin D_2, E_2.

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Since 1964 Samuelsson[1] succeeded in extracting prostaglandin (PG) from cow brain, extensive biochemical and neurochemical studies have been conducted on many

types of PGs in the central nervous system of various mammals. Hayaishi *et al.*[2-6] reported that PGD_2 affects the preoptic area of the anterior hypothalamus as a sleep-inducing substance and PGE_2 acts on the posterior hypothalamus as a wakefulness-inducing substance. Therefore the physiological actions of these PGs on the anterior and posterior hypothalamus are essential for the regulation of the sleep-wakefulness cycle and possibly for the control of consciousness.

Although there are important physiological differences between natural sleep and general anaesthesia, elucidation of the roles of PGD_2 and PGE_2 in the hypothalamus may clarify the mechanisms of general anaesthesia, particularly the loss of consciousness during general anaesthesia. However, there are few reports that have focused on this issue. We aimed to study the effect of isoflurane anaesthesia on the hypothalamic PGD_2 and PGE_2 contents in rats.

Methods

This study was approved by the animal experiment committee of the institution and the special permission was obtained for the decapitation of 42 conscious rats.

We used 63 Wistar male rats weighing 230–280 g (Japan Clea). They were housed in a 12 hr light and 12 hr dark environment at a temperature of 24 ± 0.5°C. They were freely fed with food and water and were acclimatized tenderly for at least a week before the experiment. All experimental procedures, including anaesthesia and decapitation, were conducted on a solitary rat between 1000 and 1500 hr by means of circadian rhythms.

The rats were divided equally into control, isoflurane, and recovery groups. Rats in the control group were placed in a plastic box of 42 cm × 27 cm × 19 cm for 30 min into which air was administered at a rate of 4 $L \cdot min^{-1}$. Then, they were decapitated. Rats in the isoflurane group were placed in the box and isoflurane 2% in air was administered at a flow rate of 4 $L \cdot min^{-1}$ for 30 min. At the end of the isoflurane inhalation they were decapitated. Isoflurane was vaporized with a calibrated vaporizer Forawick (Muraco). Isoflurane, oxygen and CO_2 concentrations were continuously monitored with Capnomac (Datex) throughout the experiment. Rats in the recovery group were anaesthetized with isoflurane 2% according to the above technique, then they were allowed to recover spontaneously in the box from which isoflurane was washed out completely. After making sure that they had recovered adequately from isoflurane anaesthesia, as judged by their righting reflex and behaviour 30 min after the end of isoflurane inhalation, they were decapitated. Rectal temperature was maintained at 37.0 ± 1°C with a heating pad during anaesthesia. To minimize stress decapitation took only one second. Immediately after decapitation the heads were frozen in liquid nitrogen and stored at −80°C until removal of the brains and measurement of PG concentrations.

The frozen rat heads were thawed by immersing in ethanol at 0°C for 30 min and, then, the brains were removed. The hypothalamus was removed using the methods of Gispen[7] and Goldstein[8].

Three hypothalamic samples taken from three rats were homogenized to make a measurement.

We measured the hypothalamic contents according to methods by Hiroshima *et al.*[9] for PGD_2 and Dewitt *et al.*[10] for PGE_2. Briefly, immediately after decapitation, the heads were frozen in liquid nitrogen to prevent rapid post-mortem biosynthesis of PGs and then stored at −80°C until measurement of PGD_2 and PGE_2. The frozen heads were thawed in pure alcohol cooled with ice, and then the hypothalamic samples were taken out. After weighing the samples, three samples were homogenized together in a mixed solution of 0.1 M perchloric acid, 0.02 M sodium EDTA and 0.1 mM sodium hydrogen sulphate. After centrifuging at 15,000 rpm for 20 min the supernatant was filtered with Model UFC2LGCOO filter (Nippon Milipore) and then it was again centrifuged at 10,000 rpm for 40 min. The obtained sample was frozen and stored at −80°C until measurement. Hypothalamic contents of PGD_2 and PGE_2 were determined according to enzyme immunoassay (Cayman chemical). The PGD_2 was measured as PGD_2-methoxamine, a derived form from hydrochloric acid methoxamine. The recovery rates and coefficients of variation of the methods employed were 96% and 2.5% for PGD_2, and 81% and 4.1% for PGE_2 respectively. These figures indicated that this method of analysis was appropriate for the present study.

The data obtained are expressed as mean ± SD. One-way analysis of variance was employed for statistical analysis. $P < 0.05$ was considered significant.

Results

The PGD_2 content in the hypothalamus was 397.9 ± 226.0 $pg \cdot g^{-1}$ wet weight for the control group, 134.2 ± 41.2 $pg \cdot g^{-1}$ for the isoflurane group and 269.1 ± 124.6 $pg \cdot g^{-1}$ for the recovery group, respectively. (Figure) The hypothalamic PGE_2 contents were 381.4 ± 139.0 $pg \cdot g^{-1}$ for the control group, 183.3 ± 26.4 $pg \cdot g^{-1}$ for the isoflurane group, and 312.2 ± 96.0 $pg \cdot g^{-1}$ for the recovery group, respectively. The contents of PGD_2 and PGE_2 of the isoflurane group were lower than those of the control groups ($P < 0.05$), but there was no statistical difference in both PGD_2 and PGE_2 contents of the control and recovery groups.

Discussion

In 1980 Laychock *et al.*[11] suggested that PGD_2 had a

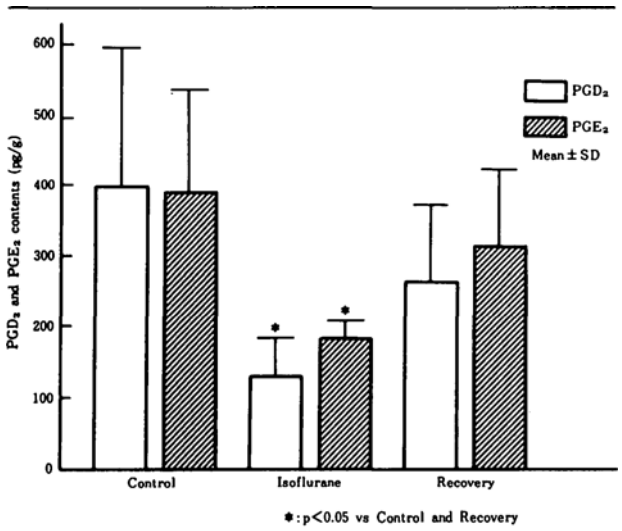

FIGURE Hypothalamic contents of PGD_2 and PGE_2 in rats. Contents of both PGD_2 and PGE_2 in the isoflurane group were lower than those in the control and recovery groups.

sedative effect and amplified the slow waves of electroencephalogram. Then Ueno *et al.*[12,13] discovered a sleep-inducing effect of PGD_2 in rats and the sleep induced by continuous PGD_2 injection could not be distinguished from natural sleep as judged by electroencephalogram, electromyogram and behavior of rats. As Yamashita[14] substantiated that PGD_2 binding protein and PGD_2 receptor were most densely distributed in the preoptic area, the site of action of PGD_2 is likely at the preoptic area which is also thought to be the sleep centre. Naito *et al.*[15] also reported that the duration and depth of natural sleep was markedly decreased by injection of either indomethacin or diclophenac which inhibits effect of cyclooxygenase to synthesize PGD_2 from arachidonic acid.

Prostaglandin E_2 is a structural isomer of PGD_2 and antagonizes the actions of PGD_2 in the brain. While PGD_2 decreases body temperature,[16] PGE_2 increases it.[17] Similarly, PGD_2 depresses[18] and PGE_2 stimulates the secretion of luteinizing hormone-releasing hormone.[19] Matsumura *et al.*[20,21] demonstrated that when PGE_2 was injected continuously into the rat cerebral ventricle, both slow wave sleep and REM sleep were inhibited, and this sleep inhibiting effect of PGE_2 was antagonized by AH6809, an antagonist of PGE_2. Watanabe *et al.*[22] reported that PGE_2 receptors were distributed most densely in the posterior hypothalamus in the central nervous system in monkeys.

In 1979 Abdel-Halim *et al.*[23] reported that general anaesthesia with either chloroform or pentobarbitone did not affect the PG contents of the rat brain. However, they did not consider their marked increases caused by post-mortem biosynthesis of PGs as indicated by Hiroshima *et al.*[9] Amano[24] measured the PGD_2 contents of the rat brain during anaesthesia with either ether or pentobarbitone according to the method by Hiroshima *et al.*,[9] and observed the PGD_2 contents in the whole brain decreased to 64% and 73% of the control, respectively.

De Simoni *et al.*[25] reported that serotonin metabolism in rats was depressed both in the anterior and posterior hypothalamus during natural sleep and was accelerated at the time of arousal. When the rat inhales isoflurane to loss of consciousness, as judged by loss of righting reflex and behaviour, both the synthesis of PGD_2 at the anterior hypothalamus as a sleep centre, and the production of PGE_2 at the posterior hypothalamus as an arousal centre are inhibited. Conversely, when rats awake and regain consciousness, the synthesis of PGD_2 in the anterior hypothalamus and that of PGE_2 from the posterior hypothalamus recovered to the pre-anaesthetic levels. Inokuchi and Oomura *et al.*[26] substantiated that neurotransmission in the lateral preoptic area following ventral noradrenergic bundle stimulation was inhibited by PGD_2 application. Hollingsworth *et al.*[27] reported that the action of PGD_2 in prolonging the duration of pentobarbitone anaesthesia is antagonized by p-chlorophenylalanine, a serotonin synthesis inhibitor. Bhattacharya *et al.*[28] also reported that administration of PGD_2 increases the serotonin content in the rat brain. These findings indicate that PGD_2 is closely related with serotonin metabolism in terms of sleep-wake cycle or consciousness-unconsciousness cycle.

Takahashi[29] reported that noradrenaline metabolism is depressed at the nucleus ceruleus, pons-hindbrain and hypothalamus during isoflurane anaesthesia in rats, but is increased when they recovered from the anaesthesia. These findings suggest to us that both the PGD_2 and PGE_2 contents in the hypothalamus recovered to pre-anaesthetic levels but the effect of PGD_2 would be inhibited by increased hypothalamic noradrenaline contents. Thus, the effect of PGE_2 may be manifest in the hypothalamus. The findings of the present study also suggest that PGD_2, PGE_2 and various other neurotransmitters interfere with each other to maintain consciousness, and general anaesthetics interfere with the metabolism of the neurotransmitters. Considering that traumatic injury causes an elevation of brain PGE_2 levels[10] appreciable decreases of PGD_2 and PGE_2 in this study may not be a simple reflection of general depressant effect of isoflurane.

In conclusion, we studied the effects of isoflurane anaesthesia on PGD_2 and PGE_2 contents in the rat hypothalamus. Both the PGD_2 and PGE_2 contents in the hypothalamus decreased during anaesthesia and recovered almost to the control levels when the rats awoke

from isoflurane anaesthesia. These findings suggest that hypothalamic PGD_2 and PGE_2 may play roles in the mechanism of loss of consciousness as judged by righting reflex and behaviour during isoflurane anaesthesia.

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