Brain cyclic nucleotide and energy metabolite responses to subanesthetic and anesthetic concentrations of halothane[1]

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Summary. Adults rats were exposed to 0.5, 1.0 and 1.5% halothane, delivered in air, for 1 h. Whole brain 3′,5′-cyclic adenosine monophosphate (cAMP) of halothane-exposed rats showed only a slight increase relative to control values. 3′,5′-Cyclic guanosine monophosphate (cGMP) was increased significantly in halothane-exposed rats, and the response was directly related to the halothane concentrations. Adenosine triphosphate (ATP) and phosphocreatine (PC) remained unchanged relative to control values. Correspondence of these values to apparent discrepancies in the literature is discussed.

Numerous investigations have shown various roles of cyclic nucleotides in nervous tissue, as well as in other tissues. In addition, cyclic nucleotides, especially cAMP, appear involved in synaptic transmission[3]. The response of brain 3′,5′-cyclic adenosine monophosphate (cAMP) to halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) anesthesia has also been previously investigated; however, results have differed in each case. Dedrick et al.[4] report 2-fold increase in rat whole brain cAMP following administration of halothane (1.0–1.5%). In contrast, Nahrwold et al.[5] obtained a slight dose-dependent reduction in mouse brain cAMP following halothane administration. Because the disparity between these observations may be due in part to species difference, the present study using rats was carried out with methods similar to those used for mice by Nahrwold et al.[5].

Materials and methods. All rats were adult, of the Long-Evans strain obtained from Charles River Co. (Boston, MA). During the course of the experiments, groups of rats were placed into plastic cages measuring 20×40×16 cm, which were located in fume hoods. Control rats were exposed only to the environment of the cage and air. Experimental rats were exposed to an identical environment; however, air passing through the cage contained halothane (U.S.P., Halocarbon Laboratories, Inc., Hackensack, NJ) in different concentrations. Halothane concentrations were maintained with a Fluotec MKII regulator, essentially as we described previously[6].

Control and halothane-exposed rats were killed by decapitation, with the head falling immediately into liquid nitrogen. Frozen heads were stored at −80 °C. Brains were dissected at a temperature of < −30 °C, maintained by the presence of blocks of dry ice in a protected dissection area.

Each brain sample consisted of about ¼ to ⅓ g of frozen tissue (mostly cortex), which was placed in a glass homogenizing tube (< 0 °C) containing 2 ml of 0.3 N perchloric acid (1.0 mM ethylenediaminetetraacetate). The sample was fixed by homogenization in the acid and centrifuged (4 °C, 5000×g for 15 min). The precipitate was dissolved in 1N NaOH for protein determination[7]. The supernatant was neutralized with 3N $KHCO_3$ (approximately 0.2 ml) and centrifuged to remove the potassium perchlorate precipitate. An aliquot (1.5 ml) of the supernatant was used to measure cyclic nucleotides, and 20–40 µl aliquots were used to measure adenosine triphosphate (ATP) and phosphocreatine (PC).

The supernatant for cyclic nucleotide assay[8] was applied to a 0.5×5.0 cm Dowex formate column (Biorad AG 1-X8, 200–400 mesh), preequilibrated with 0.1N formic acid. The column was subsequently washed once with 5.0 ml of 0.1N formic acid. Column bound cAMP was eluted in 10 ml of 2N formic acid, followed by washing the column with an additional 4.0 ml of 2N formic acid. 3′,5′-Cyclic guanosine monophosphate (cGMP) was then eluted with 10 ml of 4N formic acid. Eluates containing cAMP and cGMP were frozen, freeze-dried, and dissolved in 2.0 ml and 200 µl, respectively, of 0.05 M sodium acetate, pH 6.2. Radioimmunoassays (Becton Dickinson radioimmunoassay kits) for cyclic nucleotides were performed on duplicate 50 µl aliquots of sample by the method of Steiner et al.[9]. Values are reported as p moles/mg protein.

ATP and PC were determined by the fluorometric methods of Lowry and Passonneau[10], using a Ratio Fluorometer-2. Values are reported as n moles/mg protein.

Results and discussion. cAMP: Rats administered halothane in air for 1 h had a slight (statistically not significant) dose-dependent increase in whole brain cAMP (table). Mean values were 9, 20 and 30% greater than control values (9.2 p moles/mg protein) following administration of 0.5, 1.0 and 1.5% halothane, respectively. These results are not greatly different from those of Nahrwold et al.[5] which show little or no change in cAMP at concentrations of up to 1.5% halothane. (They had a marked decrease, particularly in cortex, at concentrations of 2% or greater.) These results differ greatly from those of Dedrick et al.[4] which show a 2-fold increase in rat brain cAMP following administration of from 1.0 to 1.5% halothane in oxygen.

The similarity of our cAMP values and the cAMP response to halothane, using rats, with the data of Nahrwold et al.[5], using mice, appears to eliminate species variation as a reason for the different results obtained by Dedrick et al.[4], who also used rats.

Control cAMP values are comparable in all these studies (in Dedrick et al.[4] the values of about 1.3 n moles/g wet wt converts approximately to 13 p moles/mg protein). Since the present results were obtained after 1 h of halothane administration and those of Nahrwold et al.[5] were obtained after only 15 min, the duration of anesthesia also does not appear a relevant variable in the present comparisons. However, the use of oxygen, as opposed to air, as the halothane vehicle may account for the significant increase in cAMP observed by Dedrick et al.[4]; although it is not readily apparent how this difference would alter the cAMP responses to halothane. In this regard it may be relevant that Woo et al.[11] observed an increase in dopamine sensi-

Halothane (H)* exposure	pmoles/mg protein cAMP	cGMP	nmoles/mg protein ATP	PC
Control	9.2±0.9	0.14±0.005	22±1.3	28±1.2
0.5% H	10±0.9	0.20±0.015	24±1.4	30±0.25
% change**	+9 (ns)	+43 (p<0.02)	+9 (ns)	+7 (ns)
1.0% H	11±0.50	0.22±0.025	26±2.2	30±1.1
% change**	+20 (ns)	+57 (p<0.02)	+18 (ns)	+7 (ns)
1.5% H	12±0.53	0.24±0.015	25±0.70	30±1.4
% change**	+30 (p<0.05)	+71 (p<0.001)	+14 (ns)	+7 (ns)

Values are mean±SE (n=3). * Administered in air for 1 h; ** % change relative to control values, p-values based on Student's t-test for 2 means (ns = not significant).

tive adenylate cyclase activity in rat caudate nucleus during halothane anesthesia in oxygen. The present halothane response and that of Nahrwold et al.[5] was obtained using air as the halothane carrier. Nevertheless, the measurement of cAMP is known to be dependent on tissue fixation[12] and subsequent handling, and consequently some other variable of the experimental procedures that is not readily apparent may yet account for the different results observed above.

cGMP: Rats administered halothane in air for 1 h exhibited a highly significant, dose-related increase in cGMP values above the control value (0.14 p moles/mg protein). This result for whole brain appears consistent with and confirms the results of Nahrwold et al.[5], who observed a several-fold increase in mouse cortex cGMP after administration of halothane in concentrations of 1% or more. The smaller response observed here for whole brain is consistent with relative weight contributions of different parts of the brain, and the marked decreases in cGMP observed[5] in cerebellum. The absolute values of cGMP are comparable to those of Dinnendahl[14], but somewhat lower here than those of Nahrwold et al.[5]. Differences in cGMP values may in part result from regional differences in samples or from post-decapitation anoxia[4,13]; however, constant levels of ATP and PC in these same samples (see below) would appear to rule out any major effect of anoxia. These metabolites decrease in response to anoxia[5,12].

Energy metabolites. Whole brain ATP and PC remained essentially unchanged (the slight increases are not statistically significant) from control values of 22 and 28 n moles/mg protein, respectively, following halothane administration. These control values and the halothane responses are comparable to results obtained by others[4,5,15].

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Anthocyanins in iris flowers

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Summary. 6 kinds of anthocyanin have been found in the flowers of 5 iris species. They were identified as the 3-p-coumaroyl-rutinosido-5-glucosides and the 3-rutinosido-5-glucosides of malvidin, petunidin and delphinidin. The distribution pattern of the iris-flower pigments is discussed; it shows that *Iris* species belonging to the section Apogon differ from the plants of the sections Xiphium and Eupogon owing to the occurrence of malvidin glycosides in addition to delphinidin glycosides.

In Japan, there are 7 *Iris* species including 5 varieties and 2 forms[2], and hundreds of *I. ensata* var. *hortensis* garden varieties have been produced by Japanese horticulturists. They have been mainly bred in 3 distinct regions: Tokyo (old district name: Edo), Kumamoto (Higo) and Mie (Ise). In the preceding paper[3], the author showed that 90 *Iris* varieties of the so-called 'Higo' type which were examined could be divided into 3 types; A (79 varieties), B (10) and C (1) on the basis of the distribution pattern of the glycosides consisting of 11 kinds of anthocyanins. In order to provide a comparison with those experimental results, the author has carried out the present study on the flower anthocyanins of 4 wild species and also *I. tectorum* which was originally distributed in China.

Flower anthocyanins in 1% methanolic HCl extract were separated into several bands by paper chromatography in n-BuOH-HCl-H_2O (7:2:5). 6 pigments were identified: the 3-p-coumaroylrutinosido-5-glucoside of malvidin (MP), petunidin (PP) and delphinidin (DP), and the 3-rutinosido-5-glucosides of malvidin (M), petunidin (P) and delphinidin (D). 3 of these, MP, PP and DP, have hitherto been found as ensatin[3], petanin[4] and delphanin (= violanin)[5], respectively. R_f values were 0.45 (MP), 0.48 (PP), 0.37 (DP), 0.17 (M), 0.15 (P) and 0.10 (D). Each band was further purified by paper chromatography in HOAc-HCl-H_2O (3:1:8) (R_f values were 0.78, 0.73, 0.68, 0.80, 0.74 and 0.53, respectively). The ratio of individual anthocyanins in the flower extracts were estimated by a Tôyô Digital Densitorol DMU-33C (500 nm filter) using the paper chromatograms. The identification of the separated pigments was carried out as described earlier[6] and also by spectral and chromatographic comparison with authentic specimens. The anthocyanin distribution in the plants was as follows; Apogon section - *I. ensata* var. *spontanea:* MP (5)[7], PP (3), M (1), P (0.5), delphinidin glycoside (÷D) (0.5); *I. laevigata:* MP (3.5), PP (4.5), M (0.5), P (1), (÷D) (0.5); *I. sanguinea:* DP (6), PP (3), D (0.9), P (0.1), malvidin glycoside (trace). Evansia section - *I. japonica:* DP (5.5), PP (4), D (0.4), P (0.1), malvidin glycoside (trace); *I. tectorum:* DP (10), D (trace). Anthocyanins of both *I. ensata* var. *spontanea* and *I. laevigata* were very similar to those of A type of the 'Higo' varieties containing ensatin as the major pigment, but *I. sanguinea* and *I. japonica* were different from any of the 3 'Higo' types, because they contained only a trace amount of malvidin glycoside. According to Ueno et al.[8], *I. setosa* contains ensatin and a trace amount of delphinidin