Decreased histamine synthesis in the rat brain by hypnotics and anaesthetics

Although increasing evidence points to a possible neurotransmitter function for cerebral histamine, surprisingly, its level seems to be little affected by a variety of psychotropic drugs (see the reviews by Green, 1970; Snyder & Taylor, 1972). However, since considerable modification in the turnover rate of cerebral amines can occur without altering their endogenous levels (Glowinski & Baldessarini, 1966), we have studied effects of several hypnotics and anaesthetics on the synthesis of [^{3}H]histamine from labelled [^{3}H]histidine administered intraventricularly. This route of administration of the [^{3}H]precursor was chosen to obtain sufficient labelling of cerebral histamine which has an endogenous level approximately one-tenth that of noradrenaline or 5-hydroxytryptamine. Both the regional and subcellular distributions of [^{3}H]histamine synthesized in this way were similar to those of the endogenous amine (Pollard, Bischoff & Schwartz, unpublished); this specificity of the labelling process probably results from the fact that brain histamine synthesis depends on a specific decarboxylase (Schwartz, Lampart & Rose, 1970), apparently localized in the cytoplasm of nerve-endings (Baudry, Martres & Schwartz, 1973). Additionally, a conversion index, calculated as the ratio of [^{3}H]histamine to the specific activity of the precursor (Sedvall, Weise & Kopin, 1968) was found to increase linearly with time up to 50 min, thus indicating a constant rate of amine synthesis during this period, and that it was probably little affected by the short anaesthesia used for intraventricular injection.

Groups of 6–7 Sprague-Dawley male rats (200 $\pm$ 20 g) were treated with one of the following drugs: sodium pentobarbitone (35 mg kg^{-1}, i.p.), sodium phenobarbitone (130 mg kg^{-1}, i.p.), chloral hydrate (350 mg kg^{-1}, i.p.) or a mixture of oxygen and methoxyflurane (approximately 3% administered at a rate of 1·5 litre min^{-1} through a special mask from the anaesthesia device of Société Minerve, Courbevoie, France). As soon as the rats lost their righting reflex, they were injected intraventricularly with 20 μl of artificial cerebrospinal fluid (Merlis, 1940) containing 60 μCi of 2,5-[^{3}H]L-histidine (spec. act. 50 Ci mmol^{-1}, the Radiochemical Centre, Amersham). They were then kept at an ambient temperature of 30° to maintain normothermia, and killed 10 min later whilst still sedated (the group treated with methoxyflurane was administered anaesthetic at a 1% concentration during the whole 10 min period).

The controls received the intraventricular injection under light anaesthesia by a mixture of O_2 (66%)–N_2O (33%)-methoxyflurane (1%), from which they recovered in less than 2 min; they were then killed 10 min after the injection. This mixture was preferred to the classical ether anaesthesia generally used for intraventricular injection (Glowinski & Axelrod, 1965) but the conversion index was essentially the same with the two methods. The whole brain was homogenized in 0·4N $HClO_4$ (6 ml g^{-1}) and the acid extract analysed for [^{3}H]histamine and [^{3}H]methylhistamine by ion-exchange chromatography followed by isotopic dilution or solvent extraction (Schwartz, Pollard & others, 1971; Schwartz, Rose & Caillens, 1973). Endogenous histamine and L-histidine were assayed spectrofluorometrically, the latter after decarboxylation under the action of a bacterial enzyme (Schwartz, Lampart & Rose, 1972). [^{3}H]Histidine was also specifically assayed by decarboxylation followed by chromatographic isolation of [^{3}H]histamine (Schwartz & others, 1973).

As indicated in Table 1 by the significantly lower values of the conversion index, [^{3}H]histamine synthesis was decreased by 42 to 56% in the animals anaesthetized during the 10 min experimental period, except in those under methoxyflurane.

Table 1. *Effect of different hypnotics and anaesthetics on the formation of [^3H] histamine and [^3H]methylhistamine in the rat brain.* [^{3}H]histidine (60 μCi per 20 μl) was injected intraventricularly 10 min before decapitation. Each value is the mean + s.e. of 6–7 determinations. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Treatment	[^{3}H]Histamine d min^{-1} mg^{-1}	[^{3}H]Methylhistamine d min^{-1} mg^{-1}	Conversion index (1)
Controls	17·1 ± 2·0	14·7 ± 0·8	5·5 ± 0·7
Pentobarbitone	10·1*** ± 1·1	5·7*** ± 1·3	2·9** ± 0·3
Phenobarbitone	11·5*** ± 0·4	8·0*** ± 1·5	3·2* ± 0·3
Chloral	8·2*** ± 1·1	7·1*** ± 1·3	2·4** ± 0·4
Methoxyflurane	16·5 ± 2·0	10·1*** ± 0·4	5·2 ± 0·8

(1) The conversion index (Sedvall & others, 1968) is the ratio of [^{3}H]histamine (d min^{-1} g^{-1} of tissue) to the specific activity of [^{3}H]histidine (d min^{-1} ng^{-1} of amino-acid).

Additionally, in each group (including the latter, but to a lesser degree), there was a marked reduction in [^{3}H]methylhistamine (up to 61%). Since the endogenous histamine level was not modified, these results suggest that the turnover of the cerebral amine was rapidly reduced in anaesthetized animals. This view is strengthened by our data showing that another barbiturate delays the disappearance of [^{3}H]histamine: in animals treated with thiopentone 1 h after the intraventricular injection of [^{3}H]histidine (i.e. at a time when synthesis of the [^{3}H]amine was low due to the low specific activity of the remaining [^{3}H]amino-acid) and killed 3 h later, cerebral [^{3}H]histamine levels were twice those of controls (Pollard & others, unpublished; again there was no alteration of the endogenous amine level.

Barbiturates have already been shown to reduce markedly the turnover of several brain neurotransmitters: acetylcholine (Schuberth, Sparf & Sundwall, 1970), dopamine (Corrodi, Fuxe & Hökfelt, 1966), noradrenaline (Persson & Waldeck, 1971; Lidbrink, Corrodi & others, 1972), 5-HT (Corrodi, Fuxe & Hökfelt, 1967). The mechanism of action of anaesthetics is still debated, but their effect on brain amine turnover may be linked to the reduced impulse flow they induce in central neurons (Noda & Adey, 1973). Therefore, the present observations suggest a possible dependence of brain histamine turnover on nerve impulse flow, an hypothesis consistent with recent data showing that the amine is released from brain slices under depolarization by potassium (Atack & Carlsson, 1972; Snyder & Taylor, 1972). In addition, irrespective of the mechanism of action of the tested drugs, the present experiments clearly indicate that the rate of histamine turnover in brain can be strikingly modified in a few minutes.

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REFERENCES

ATACK, C. & CARLSSON, A. (1972). *J. Pharm. Pharmac.*, **24**, 990–992.
BAUDRY, M., MARTRES, M. P. & SCHWARTZ, J. C. (1973). *J. Neurochem.*, in the press.
CORRODI, H., FUXE, K. & HÖKFELT, T. (1966). *J. Pharm. Pharmac.*, **18**, 556–558.

CORRODI, H., FUXE, K. & HÖKFELT, T. (1967). *Eur. J. Pharmac.*, **1**, 363–368.

GLOWINSKI, J. & AXELROD, J. (1965). *J. Pharmac. exp. Ther.*, **149**, 43–49.

GLOWINSKI, J. & BALDESSARINI, R. J. (1966). *Pharmac. Rev.*, **18**, 1201–1238.

GREEN, J. P. (1970). *Handbook of Neurochemistry*, vol. 4, p. 221. Editor: Lajtha, A. New York: Plenum Press.

LIDBRINK, P., CORRODI, H., FUXE, K. & OLSON, L. (1972). *Brain Res.*, **45**, 507–524.

MERLIS, J. K. (1940). *Am. J. Physiol.*, **131**, 67–72.

NODA, H. & ADEY, W. R. (1973). *Brain Res.*, **54**, 243–259.

PERSSON, T. & WALDECK B. (1971). *J. Pharm. Pharmac.*, **23**, 377–378.

SCHUBERTH, J., SPARF, B. & SUNDWALL, A. (1970). *Drugs and cholinergic mechanisms in the central nervous system*, p. 117. Editors: Heilbronn, E. & Winter, A. Stockholm: Research Institute of National Defence.

SCHWARTZ, J. C., LAMPART, C. & ROSE, C. (1970). *J. Neurochem.*, **17**, 1527–1532.

SCHWARTZ, J. C., POLLARD, H., BISCHOFF, S., REHAULT, M. C. & VERDIERE, M. (1971). *Eur. J. Pharmac.*, **16**, 326–335.

SCHWARTZ, J. C., LAMPART, C. & ROSE, C. (1972). *J. Neurochem.*, **19** 801–810.

SCHWARTZ, J. C., ROSE, C. & CAILLENS, H. (1973). *J. Pharmac. exp. Ther.*, **184**, 766–799.

SEDVALL, G. C., WEISE, V. K. & KOPIN, I. J. (1968). *Ibid.*, **159**, 274–282.

SNYDER, S. H. & TAYLOR, K. M. (1972). *Perspectives in Neuropharmacology, a tribute to Julius Axelrod*, p. 43. Editor: Snyder, S. H. New York: Oxford University Press.

Application of mass spectrometry to enzyme inhibition studies

Identification of the amino-acid residue alkylated in an enzyme by an irreversible inhibitor is usually accomplished by comparison of the molar ratios of the amino-acids found in the native and inhibited enzyme. Reduction in the content of a specific amino-acid in the modified enzyme is evidence for alkylation of that residue (Lawson & Schramm, 1962; Schoellmann & Shaw, 1963; Gundlach, Stein & Moore, 1959). The alkylated residue behaves differently from the parent amino-acid on the ion-exchange column used in the analysis and can on occasions be detected on the scan and subsequently identified using a synthetic sample (Lawson & Schramm, 1962; Gundlach & others, 1959).

Amino-acid analysis is satisfactory for the detection of changes in the titres of histidine and methionine since these titres are usually low in proteins and loss of one residue in the alkylation is readily discernable. The predominance of serine and, in certain enzymes, lysine, limits the use of the method since the corresponding changes in these titres are within the limits of accuracy of the analysis. Furthermore, amino-acid analysis cannot be used with confidence to detect the site of alkylation where impure enzyme preparations are used, especially if a separation technique is used for removal of excess inhibitor since native and inhibited enzyme preparations may then have variable compositions (Al Shabibi, 1972).

Mass spectrometry is a technique which may be unambiguously applied to the identification of a minor constituent in a mixture of components by precise mass measurement of either its parent ion, M^+, or main fragmentation ion. We have shown using bovine ribonuclease and the irreversible inhibitor iodoacetate that mass spectrometry could be a useful new tool for the rapid identification of an alkylated amino-acid in a protein hydrolysate. We consider that this new method would be particularly useful for impure or degraded modified enzyme.

Ribonuclease is alkylated by iodoacetate at the active site, reaction occurring with histidine (His-119) at pH 5·5–6·0 and with lysine at pH 8·5–10·0 (Gundlach & others, 1959). Reaction between iodoacetate and ribonuclease at pH 8·5 was followed by