to severe changes in ambient temperature. It also infers that endogenous opioid peptides may have a physiological role in regulating the body temperature of rats undergoing changes in environmental temperature. Whether endorphins principally affect metabolic, cardiovascular or behavioral components of this acclimation process is not yet known.

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REFERENCES

Blasig, J., Hollt, V., Bauerle, U., Herz, A. (1978) Life Sci. 23: 2525–2532

Blumberg, H., Dayton, H. B. (1973) in: Kostelitz, H. W., Collier, H. O. J., Villarreal, J. (eds) Agonist–Antagonist Action of Narcotic Analgesic Drugs. University Press, pp 110–119

Clark, W. G., Cumby, H. R. (1978) Br. J. Pharmacol. 63: 65–71

Goldstein, A., Lowery, P. J. (1975) Life Sci. 17: 927–932

Holaday, J. W., Wei, E., Loh, H. H., Li, C. H. (1978) Proc. Natl. Acad. Sci. 75: 2923–2927

Resnick, R. B., Valavka, J., Freeman, A. M., Thomas, M. (1974) Am. J. Psychiatry 131: 646–650

Thornhill, J. A., Hirst, M., Gowdey, C. W. (1978) Can. J. Physiol. Pharmacol. 56: 483–489

Decrease of noradrenaline *O*-methylation in rat brain induced by L-dopa. Reversal effect of *S*-adenosyl-L-methionine

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S-Adenosyl-L-methionine (SAMe) is the main methyl donor in many biochemical reactions (Axelrod 1966; Salvatore et al 1977; Usdin et al 1979). In the central nervous system SAMe takes part in catecholamine metabolism (Stramentinoli & Maffei 1978) and in the synthesis of biologically important compounds, such as melatonin (Wurtman & Axelrod 1968) and phosphatidylcholine (Mozzi & Porcellati 1979; Blusztajn et al 1979).

The SAMe concentration may be depleted by such compounds as L-dopa, which may act as methyl group acceptors (Wurtman et al 1970; Ordonez & Wurtman 1973).

If SAMe concentration is critical, treatment with L-dopa may affect normal transmethylating reactions. In fact, previous studies by Chalmers et al (1971) demonstrated that acute L-dopa administration to rats intracisternally injected with [^{3}H]noradrenaline caused a depletion of brain SAMe as well as a reduction in *O*-methylated [^{3}H]noradrenaline derivatives.

Our aim has been to demonstrate that the decreased *O*-methylation of brain noradrenaline following the depletion of brain SAMe after L-dopa administration can be reversed by exogenous treatment with SAMe.

S-[Methyl-^{14}C] Adenosyl-L-methionine($^{14}CH_3$-SAMe), 55 mCi mmol^{-1}, was obtained from the Radiochemical Centre, Amersham, England; L-dopa was from Merck, Darmstadt, Germany, and SAMe as disulphate-di-*p*-toluenesulphonate was obtained from BioResearch Co., Milan, Italy.

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Male CD-COBS albino rats (Charles River, Italy), 175–200 g were injected with L-dopa (100 mg kg^{-1}, i.p.) or with L-dopa plus SAMe (100 mg kg^{-1}, i.m., 5 min after L-dopa).

L-Dopa was suspended in 2% gum arabic solution kept continuously stirred and injected in a volume of 4 ml kg^{-1}.

SAMe was dissolved in phosphate buffer 0·17 M to reach a final pH of 7·0 and injected in a volume of 2·0 ml kg^{-1}. The control rats received the corresponding vehicles; the phosphate buffer was also brought to pH 7·0 using 1 M HCl.

The animals were decapitated 45 min after SAMe injection. The brains were rapidly removed and the cortex dissected, washed carefully in chilled saline, blotted on filter paper and frozen on dry ice and kept at −30 °C until assayed (within 24 h).

The cerebral tissue was then homogenized in 10% TCA dissolved in 0·05 M HCl 1:5 (w/v). After centrifugation at 3000 *g* at 4 °C for 10 min, 1-ml aliquots of the clear supernatant were mixed with 20 μl of [methyl-^{14}C]-SAMe (10 nCi). The solution was then washed three times with 2 vol of peroxide-free ether previously saturated with 0·05 M HCl.

SAMe concentration was then assayed according to the radioenzymatic method of Baldessarini & Kopin (1966), and 3-methoxy-4-hydroxyphenylglycol-SO_4 (MHPG-SO_4), the main methoxylated metabolite of brain noradrenaline, was determined spectrofluorimetrically in cortex extracts after chromatographic separation on DEAE Sephadex A-25 columns, following the procedure of Meek & Neff (1972).

Table 1. Depletion of SAMe and MHPG-SO_4 in rat brain hemispheres induced by L-dopa and reversal effect of SAMe.

Treatment	SAMe	MHPG-SO_4
Saline	10·39 ± 0·16	0·19 ± 0·04
L-Dopa	3·48 ± 0·26[a]	0·04 ± 0·01[a]
SAMe	13·74 ± 0·34[b]	0·21 ± 0·03
L-Dopa + SAMe	5·68 ± 0·37[d]	0·17 ± 0·01[c]

Data are expressed as $\mu g\ g^{-1}$ and are the mean of 4 values for SAMe and of 6 values for MHPG-SO_4.

Significance has been determined using a Duncan's new multiple range test.

[a] $P < 0{\cdot}01$ vs controls. [b] $P < 0{\cdot}05$ vs controls. [c] $P < 0{\cdot}01$ vs L-dopa. [d] $P < 0{\cdot}05$ vs L-dopa.

In agreement with the results of Wurtman et al (1970) and Ordonez & Wurtman (1973), L-dopa treatment elicited a drastic drop in SAMe concentration (Table 1). This decrease might be due to enhanced methyl group utilization for the *O*-methylation of L-dopa itself or of dopamine and its deaminated metabolites (Hornykiewicz 1966). This seems to happen at the expense of other transmethylation reactions. At the same time, the *O*-methylation of noradrenaline is also drastically reduced (Table 1).

We had previously shown that it is possible to increase SAMe concentration in the brain by exogenous administration of this cofactor both intravenously (Stramentinoli et al 1977) and intramuscularly (Stramentinoli et al 1978). In agreement with those previous results, the concentration of the cofactor was raised by 32% in rats treated intramuscularly with a dose of 100 mg kg^{-1} SAMe (Table 1); in the animals where SAMe concentration had been depleted by L-dopa treatment, intramuscular administration of the cofactor at 100 mg kg^{-1} can induce a 63% increase of endogenous SAMe concentrations. Although this dose was not enough to compensate completely for the reduced concentration of SAMe in the brain after L-dopa administration, it was sufficient to restore the concentration of MHPG-SO_4 to normal values, thus showing that the decrease of this metabolite is related to a deficiency in methyl groups.

These results also imply that the lowering of brain SAMe concentration after L-dopa administration may affect the methylation of other compounds that are the substrates for methylating enzymes. This could directly or indirectly influence the normal function of certain neuronal systems. For instance, although the mechanism of action is still obscure, we have found that SAMe enhances 5-HT and noradrenaline synthesis (Curcio et al 1978; Algeri et al 1979).

Recently, studies carried out using erythrocyte and reticulocyte membranes have shown that the membrane phospholipid methylation plays an important role in different cellular events (Hirata et al 1979). It is of particular interest, among them, the relation existing between membrane phospholipid methylation and the coupling of β-receptors with adenylate cyclase (Hirata et al 1979). If this mechanism is present also in the nervous system, the interaction between neurotransmitters and receptors may be possibly affected by a deficiency of SAMe.

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REFERENCES

Algeri, S., Catto, E., Curcio, M., Ponzio, F., Stramentinoli, G. (1979) in: Zappia, V., Usdin, E., Salvatore, F. (eds) Biochemical and Pharmacological Roles of Adenosylmethionine. Pergamon Press Ltd, Oxford, p 81

Axelrod, J. (1966) Pharmacol. Rev. 18: 95–113

Baldessarini, R. J., Kopin, I. J. (1966) J. Neurochem. 13: 769–777

Blusztajn, J. K., Zeisel, S. H., Wurtman, R. J., Brain Res. in the press

Chalmers, J. P., Baldessarini, R. J., Wurtman, R. J. (1971) Proc. Natl. Acad. Sci. 68: 662–666

Curcio, M., Catto, E., Stramentinoli, G., Algeri, S. (1978) Prog. Psychopharmacol. 2: 65–71

Hirata, F., Axelrod, J., Strittmatter, W. J. (1979) in: Usdin, E., Borchardt, R. T., Creveling, C. R. (eds) Transmethylation. Elsevier North Holland Inc., New York, p 233

Hornykiewicz, D. (1966) Pharmacol. Rev. 18: 925–964

Meek, J. L., Neff, N. H. (1972) Br. J. Pharmacol. 45: 435–441

Mozzi, R., Porcellati, G. (1979) FEBS Lett. 100: 363–366

Ordonez, L. A., Wurtman, R. J. (1973) Biochem. Pharmacol. 22: 134–137

Salvatore, F., Borek, E., Zappia, V., Williams-Ashman, H. G., Schlenk, F. (1977) The Biochemistry of Adenosylmethionine. Columbia University Press, New York

Stramentinoli, G., Catto, E., Algeri, S. (1977) Commun. Psychopharmacol. 1: 89–97

Stramentinoli, G., Catto, E., Algeri, S. (1978) in: Andreoli, V. M., Agnoli, A., Fazio, C. (eds) Transmethylations and the Central Nervous System. Springer-Verlag, Berlin, p 111

Stramentinoli, G., Maffei, F. (1978) in: Andreoli, V. M., Agnoli, A., Fazio, C. (eds) Transmethylations and the Central Nervous System. Springer-Verlag, Berlin, p 49

Usdin, E., Borchardt, R. T., Creveling, C. R. (1979) Transmethylation. Elsevier/North Holland Inc., New York

Wurtman, R. J., Axelrod, J. (1968) Adv. Pharmacol. 67: 141–150

Wurtman, R. J., Rose, C. M., Metthysse, S., Stephenson, J., Baldessarini, R. (1970) Science 169: 395–397