Serotonin and its Metabolites; their Respective Roles in the Production of Hypothermia in the Mouse

FELDBERG and MYERS[1] have proposed a critical role for the biogenic amines in the CNS control of thermoregulatory responses. Both intraperitoneal and intraventricular injections of serotonin (5-HT) produce a drop in body temperature in the mouse[2]. We now report a series of experiments in which we studied the effect on body temperature of the serotonin metabolites, 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptophol. Tryptophol[3] and 5-methoxytryptophol were also studied to evaluate the specificity of 5-HT metabolites in the production of body temperature changes.

Methods and procedures. 90 random bred albino mice (CD-1 strain; weight, 21–28 g) were assigned, 4 to a group, to each condition of 5 experiments. Rectal temperatures were measured with a thermistor inserted 1.5 cm beyond the anal sphincter and recorded by a Digitec digital thermometer calibrated to 0.01 °C[4]. Agents were administered i.p. at 0.01 ml/g of body weight in each experiment. Doses of tryptophol (100, 200, and 400 mg/kg), 5-methoxytryptophol (200, 400, and 600 mg/kg), and 5-hydroxytryptophol (800 and 1200 mg/kg), were each prepared in propylene glycol[5]. The 5-HIAA was prepared in buffered sterile saline and administered at doses of 250, 500 and 1000 mg/kg[6]. Appropriate vehicle controls were also included (Figure 1).

In order to evaluate the respective roles of 5-HT and its metabolites in the production of hypothermia, the effect of MAO inhibition on a 5-hydroxytryptophan (5-HTP)-induced hypothermia was also studied[7]. Groups of 16 mice were pretreated with either MAO inhibitor (25 mg/kg of phenelzine dissolved in sterile saline) or placebo. 1 h later each pretreatment group was divided into 4 groups of 4 mice each and injected with either a steriod suspending vehicle[8], or 50, 100 or 200 mg/kg of 5-HTP dissolved in this vehicle (Figure 2).

Results and discussion. Serotonin, formed from 5-HTP, is metabolized to 5-hydroxyindoleacetaldehyde, which can then be metabolized to 5-hydroxytryptophol in a

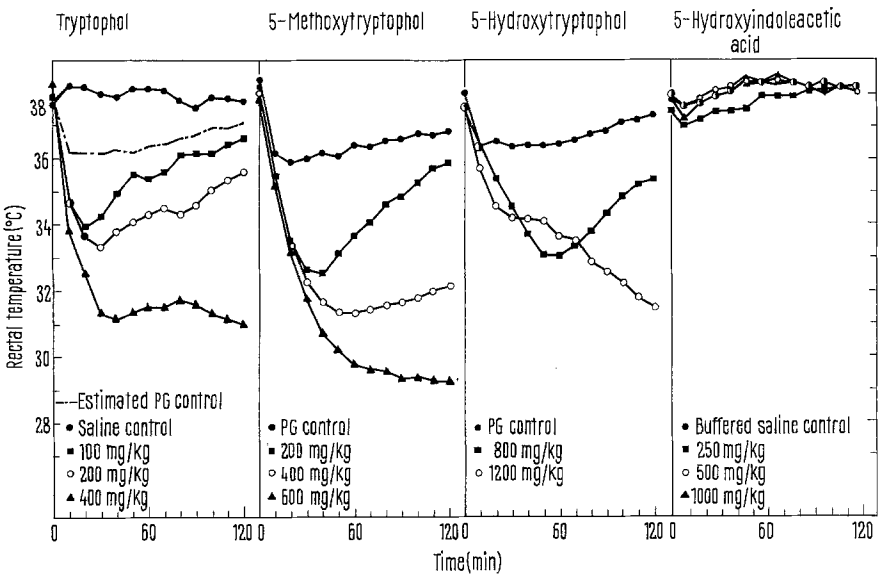

Fig. 1. Rectal temperature measures were recorded before and every 10 min after administration of drugs for a total of 2 h. The mean rectal temperature for each group of mice is shown as a function of drug, dose and time. The estimated propylene glycol control (stippled line in Panel 1) was calculated from the PG control data for the 5-methoxytryptophol and 5-hydroxytryptophol experiments. The half-filled circles indicate overlapping data points.

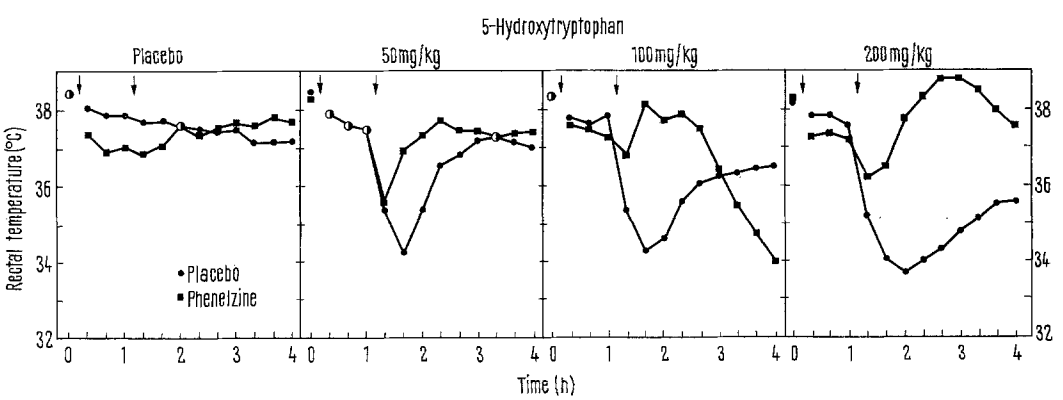

Fig. 2. Rectal temperature measures were recorded before, and every 20 min after administration of drugs for a total of 4 h. The mean rectal temperature for each group of 4 mice is shown as a function of pretreatment (the time of administration of 25 mg/kg of phenelzine or a placebo is marked by the first arrow in the figure), dose of L-5-hydroxytryptophan (marked by the second arrow), and time. The half-filled circles indicate overlapping data points.

[1] W. FELDBERG and R. D. MYERS, J. Physiol., Lond. *173*, 226 (1964).
[2] R. T. BRITTAIN and S. L. HANDLEY, J. Physiol., Lond. *192*, 805 (1967). – F. N. FASTIER, R. N. SPEDEN and H. WAAL, Br. J. Pharmac. *12*, 251 (1957).
[3] The term 'tryptophols' will be used to refer to 5-hydroxytryptophol, 5-methoxytryptophol as well as tryptophol.
[4] Rectal temperatures were recorded by the method of BROWN; A. M. BROWN, J. Pharm. Pharmac. *16*, 701 (1964). Room temperature varied between 23–26 °C.
[5] Propylene glycol and water, in a 1:1 mixture was used as the vehicle.
[6] The pH of the 5-HIAA injections were neutral at the 250 and 500 mg/kg dose and slightly acid (pH of 6) at the 1000 mg/kg dose.
[7] J. KARJA, N. T. KARKI and E. TALA, Acta pharmac. tox. *18*, 255 (1961).
[8] The Steriod Suspending Vehicle made by Armour Inc. was used.
[9] A. FELDSTEIN and O. WILLIAMSON, Br. J. Pharmac. *34*, 38 (1968).
[10] A separate 2 factor (dose and time) repeated-measure analysis of variance was performed on the 12 post-injection rectal temperature measures for each agent. B. J. WINER, *Statistical Principles in Experimental Design* (McGraw-Hill, New York 1962), p. 298.
[11] The 'tryptophols' dissolved in sesame oil also produced significant drops in body temperature. Sesame oil, itself, did not effect body temperature.
[12] The data were analyzed, in each case, by a three-way repeated-measure analysis of variance (B. J. WINER, *Statistical Principles in Experimental Design* (McGraw-Hill, New York 1962), p. 298). The rectal temperature measures just prior to and 20 min following administration of 5-HTP were compared and revealed that pretreatment with the MAO inhibitor did not differentially affect the initial response of the animals to 5-HTP. There was also no evidence in the data that the maximum rectal temperature following 5-HTP was significantly greater than the initial predrug rectal temperature. Some of the MAO-inhibited animals, however, experienced hyperthermia (particularly at the 200 mg/kg dose of 5-HTP) but variability existed at each dose level. An overall analysis of variance, which included all the data, revealed a significant triple interaction ($P < 0.01$); that is, rectal temperature varied as a function of pretreatment, dose of 5-HTP and time since injection.
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[18] H. C. SABELLI, W. J. GIARDINA, S. G. A. ALIVISATOS, P. K. SETH and F. UNGAR, Nature *223*, 73 (1964).
[19] Supported by NIMH Training Grant No. T01 MH-10625 and NIMH Grant No. MH-13540.

reversible reaction or to 5-HIAA in an essentially irreversible reaction[9]. Figure 1 shows that 5-hydroxytryptophol or its analogues, tryptophol and 5-methoxytryptophol, produced a significant ($P < 0.01$) dose and time dependent decrease in body temperature, but that 5-HIAA had no effect on body temperature[10,11]. Figure 2 shows that pretreatement with an MAO inhibitor prevented the 5-HTP-induced hypothermia, suggesting that the 'tryptophol' metabolite rather then 5-HT itself was the active hypothermic agent. The small initial drop in rectal temperature (Figure 2) which occurred immediately after administration of 5-HTP to the MAO-inhibited animals[12] could be accounted for by depletion of norepinephrine by 5-HTP[13]. The subsequent increase in body temperature, however, did not lead to a significant hyperthermia[12].

Two issues are raised when large doses of drugs are given i.p.; first whether the observed effects are of central or peripheral origin, and second whether the observed effects are pharmacological or physiological in nature. The available evidence suggests that the 'tryptophols' produce CNS effects[14]. The doses of the 'tryptophols' were specifically selected from a range that produced a dose-dependent sleep response[15]. The 'tryptophols', as found for 5-methoxytryptophol[16], may not readily cross the blood-brain barrier, therefore, requiring large doses. They are also known to be rapidly metabolized by the liver[17], although the enzymes required to metabolize the 'tryptophols' are found in various areas of the brain[14]. Together, these data make tenable the working hypothesis, that the observed effects represent changes in a centrally mediated physiological regulatory process.

The formation of the 'tryptophols' from the aldehyde is a reversible process, and since 5-hydroxyindoleacetaldehyde has CNS effects[18], a possible role for the aldehyde-metabolites in the production of hypothermia must be evaluated. Irrespective of which metabolite is active, the results of the present experiments make imperative consideration of the metabolites in any 5-HT induced modification of body temperature, and raise doubts about 5-HT itself being the active agent[19].

Zusammenfassung. In Mäuseversuchen wurde gezeigt, dass nicht Serotonin, sondern zwei seiner Metaboliten (5-Hydroxytryptophol oder dessen Ausgangsstoff 5-Hydroxyindolacetaldehyd) die Hypothermie erzeugen.

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Kristalloide Einschlüsse im Karyoplasma von Spermatiden eines Myriapoden

Bei der Untersuchung der Spermiohistogenese von Spirostreptus spec. (Diplopoda, Myriapoda)[1] fielen langgestreckte osmiophile Körper im Karyoplasma auf, die eine sehr regelmässige Streifung besitzen (Figur).

Die Periode der Querstreifung beträgt zwischen 120–135 Å, die osmiophilen Streifen sind 75–85 Å breit, die hellen 40–50 Å. Sie entspricht einem regelmässigen Aufbau der Einschlüsse aus Lamellen. Die Einschlüsse sind meist langgestreckt, stabförmig und bis 10 µ lang bei einer Breite von 0,1 µ; es kommen aber auch gedrungene, 0.25 µ breite Körper vor. Bemerkenswert ist die Nachbarschaft zu einer filamentösen Struktur, die in derselben Richtung wie die Längsachse des kristalloiden Einschlusses verläuft (Figur, b).

Der Körper tritt in einer bestimmten Phase der Spermiohistogenese auf, nämlich nachdem die Anlage des Akrosomkomplexes abgeschlossen ist und der Komplex nur noch an Grösse zunimmt, und bevor die Kernkondensation einsetzt (vergl.[1]). Im Kern konnte nie mehr als ein Einschluss gefunden werden, im Zytoplasma kommen vergleichbare Einschlüsse nicht vor.

[1] E. HORSTMANN und H. BREUCKER, Z. Zellforsch. *99*, 153 (1969).