## Mediation of the Hypoglycaemic Effect of 5-Hydroxytryptophan by a Central Nervous System Action

L-5-hydroxytryptophan (5HTP) produces hypoglycaemia in mice pretreated with monoamineoxidase inhibitors (MAOI)[1,2]. This effect appears to be mediated by 5-hydroxytryptamine (5HT). FURMAN[2] suggested that the response may be due to the accumulation of 5HT in the central nervous system (CNS) since marked effects of central origin, known to be produced by 5HT (head twitching, tremor), accompanied the hypoglycaemic response. Moreover, 5HT, which does not penetrate into the CNS when administered i.v., did not produce hypoglycaemia. More information concerning this response is presented in this paper.

*Methods.* Male albino mice (25–30 g) starved overnight were used. All animals received nialamide (80 mg/kg i.p.) 20 h and 2 h prior to blood sampling; i.v. injections were made into a tail vein. Intracerebroventricular injections were made in a volume of 10 µl according to the method of HALEY and MCCORMICK[3], using a 26 g × 1 cm needle. The effective length of the needle was reduced to 3 mm by inserting it through a tightly fitting PTFE sleeve of external diameter 1 cm.

Mice were lightly anaesthetised with ether just prior to blood sampling and blood was removed from the femoral vein. One sample only was removed from each mouse. The blood was immediately centrifuged (Beckman Microfuge) and 0.1 ml plasma removed for glucose determination. Glucose was measured using a microcolorimetric copper reduction technique[4].

Where 2 treatments were involved (i.e. drug solution and control vehicle) each was administered alternately to mice selected at random. Where 4 treatments were used (e.g. pentobarbitone + vehicle for 5HTP; pentobarbitone + 5HTP; vehicle for pentobarbitone + 5HTP; vehicle for pentobarbitone + vehicle for 5HTP) the treatments were randomized over the time period of the experiment. Statistical significance was assessed using Student's $t$-test and significance accepted where $p < 0.05$.

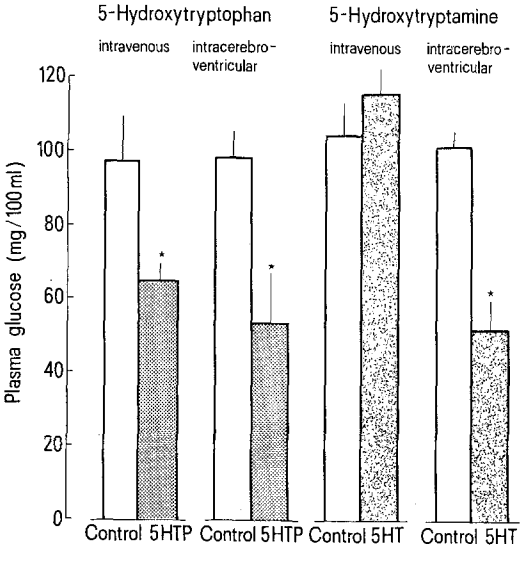


Fig. 1. Effect of 5-hydroxytryptophan (5HTP) or 5-hydroxytryptamine (5HT) on plasma glucose when injected i.v. or intracerebroventricularly into nialamide treated mice. Each column represents the mean of 10 observations. The vertical bars indicate the standard errors.
* Statistically significant difference ($p < 0.05$) between drug and control treatments.

Drugs used were L-5-hydroxytryptophan (Calbiochem), 5-hydroxytryptamine creatinine sulphate (British Drug Houses), pentobarbitone sodium (Abbott), urethane (British Drug Houses) and α-chloralose (British Drug Houses).

*Influence of route of administration.* In nialamide treated mice 5HTP (10 µg) or 5HT (10 µg) injected intracerebroventricularly each produced a hypoglycaemic response relative to saline injection (Figure 1). Additionally, marked head twitching and tremor began within minutes of the injection of 5HT or its precursor. Only 5HTP produced hypoglycaemia, head twitching and tremor when administered i.v., as reported previously[1,2].

*Effect of anaesthetics.* The administration of pentobarbitone (45 mg/kg), urethane (975 mg/kg), or chloralose (114 mg/kg), i.p. 15 min prior to 5HTP injection, prevented the hypoglycaemic responses, plasma glucose being measured 1 h after the injection of 5HTP (4 mg/kg i.v.; Figure 2). In these experiments urethane produced sedation but not anaesthesia. Neither urethane nor chloralose had any effect on the plasma glucose of fasted, nialamide-treated mice at this time. Pentobarbitone was found to have a significant hypoglycaemic effect under these conditions, thus rendering difficult the interpretation of these experiments. However the greater hypoglycaemic response to a larger dose of 5HTP (8 mg/kg) was also prevented by pentobarbitone.

*Discussion.* The ability of 5HT to produce hypoglycaemia when injected intracerebroventricularly, but not intravenously, in nialamide treated mice suggests that the hypoglycaemic response to 5HTP (seen after i.v. or i.c.v. injection) may be mediated through the accumulation of 5HT in the central nervous system. Stimulation of central 5HT receptors has been shown to inhibit the secretion of adrenocorticotrophic hormone and to increase the secretion of prolactin[5]. Both these effects could contribute towards the production of hypoglycaemia in the mouse[6,7]. The prevention of the 5HTP response by each of 3 different general anaesthetics (pentobarbitone, chloralose and urethane) may also be suggestive of a central action. These anaesthetics have been shown variously to increase the secretion of growth hormone and adrenocorticotrophic hormone[8,9] and to inhibit the secretion of prolactin[10].

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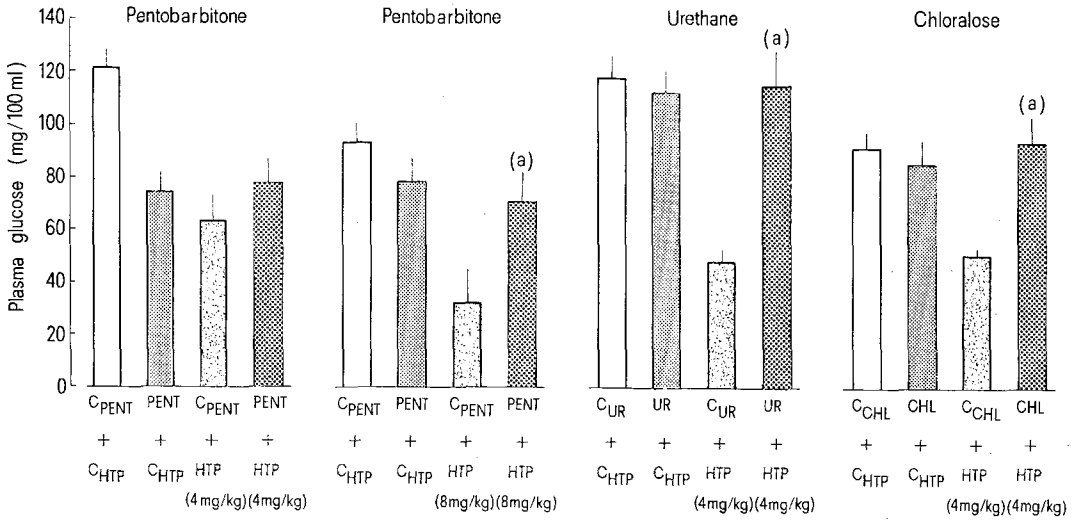


Fig. 2. Effect of pentobarbitone (PENT), urethane (UR) or chloralose (CHL) on the hypoglycaemic response to 5-hydroxytryptophan (HTP) $C_{PENT}$, $C_{UR}$, $C_{CHL}$ and $C_{HTP}$ refer to mice receiving the control vehicles for pentobarbitone, urethane, chloralose and 5-hydroxytryptophan respectively. Each column represents the mean of 12 observations. The vertical bars indicate the standard errors. (a) indicates a statistically significant difference ($p < 0.05$) between the plasma glucose found in mice receiving 5HTP and control vehicle for the anaesthetic (e.g. HTP + $C_{PENT}$), and mice receiving 5HTP and the anaesthetic (e.g. HTP + PENT).

The precise mechanisms involved in the production of hypoglycaemia by 5HTP remain to be elucidated. Furthermore, its relevance to MAOI induced hypoglycaemia as suggested by LUNDQUIST et al.[1], remains to be established.

*Résumé.* L'injection du 5-hydroxytryptophan par voie i.v. ou intracérébroventriculaire produit chez les souris l'hypoglycémie. Le 5-hydroxytryptamine y répond seulement lors d'une injection intracérébroventriculaire. Le 5-hydroxytryptophan n'a pas de réponse glycémique si les souris sont anesthétisées par du chloralose, de l'uréthane ou du pentobarbitone.

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## Acetylcholine Output into the Liquor Spaces in Conscious Dogs

Acetylcholine release into brain of conscious animals was studied only in the cerebral cortex of rabbits and cats[1–4]. But there are no reports on the acetylcholine output into cerebral ventricles and subarachnoid spaces (liquer spaces) in animals when in a conscious state. Hence, in the present study, the magnitude of the output of acetylcholine into the cerebroventricular perfusate in unanaesthetized dogs was determined.

For this purpose a Collison's cannula and a polyvinyl tube (inner diam. 1 mm and outer diam. 2.15 mm) were placed into the left lateral ventricle[5] and into the upper cervical subarachnoid space respectively[6] in dogs of both sexes (weighing 7 to 18 kg) under pentobarbitone anaesthesia (30 mg/kg) under aseptic conditions. The next day, when the dog recovers, the cerebral ventricles were perfused with sterile artificial cerebrospinal fluid[7] at a rate of 0.1 ml/min from the cannula in the lateral ventricle to cervical cannula with the help of a palmer slow injector. The outflow fluid collected at intervals of 30 min in 0.3 ml of N/3 hydrochloric acid during perfusion, was estimated for acetylcholine-like activity within 24 h on frog rectus muscle[8] and on rat blood pressure[9,10] in albino rats[11]. The absence of stimulant action on frog rectus muscle and depressant action on rat blood pressure by the perfusate, as well as standard acetylcholine (Merck) after treatment of these preparations with D-tubocurarine (Light & Co) hydrochloride and atropine hydrochloride (Merck) respectively, confirmed the true nature of acetylcholine present in the perfusate of liquor spaces in conscious dogs. In addition, some samples in alternate experiments on assay showed the same values

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