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INCREASED DOPAMINE AND SEROTONIN METABOLITES IN CSF DURING SEVERE INSULIN-INDUCED HYPOGLYCEMIA IN FREELY MOVING RATS

JABER DANGUIR*, JEAN-LUC ELGHOZI† and DOMINIQUE LAUDE†
*Laboratoire de Neurobiologie des Régulations, CNRS ER 218, Collège de France, 75231 Paris Cedex 05, France
†Laboratoire de Pharmacologie, INSERM U7, Faculté de Médecine Necker-Enfants Malades, 75730 Paris Cedex 15, France

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Abstract—The effect of insulin on dopamine (DA) and serotonin (5-HT) metabolites was determined in the cerebrospinal fluid (CSF) of the rat and compared with glucose levels in blood and CSF. CSF was continuously withdrawn from the third ventricle of freely moving rats at a constant rate of 1 μl/min. Liquid chromatography with electrochemical detection was used for the direct assay of DA and 5-HT metabolites in the CSF. The metabolites were stable during the first hour after insulin injection (6IU/Kg). A progressive increase occurred thereafter in animals which had no access to food during the time of the experiment. The maximal effect was observed 2.5 h after insulin, with respective mean increases of 80% for dihydroxyphenylacetic acid, 47% for homovanillic acid and 33% for 5-hydroxyindolacetic acid. These increases in monoamine metabolites were not observed when rats received glucose (5g/Kg ip) 45 min after insulin or when food was made available. The period for insulin-induced increase in DA and 5-HT metabolites corresponded to a maximal fall of glucose levels both in blood and CSF although the CSF glucose decrease was delayed when compared to the fall of blood glucose. The role of brain glucose and brain insulin in the control of central DA and 5-HT metabolism is discussed.

Abnormalities of cerebral oxidative metabolism and blood flow have been reported in insulin-induced hypoglycemia in humans and animals (Kety *et al.*, 1948; Ferrendelli and Chang, 1973; Lewis *et al.*, 1974; Cillufo *et al.*, 1982; Ghajar *et al.*, 1982). Dysfunction in neural transmission can be expected after peripheral administration of insulin as a consequence of a reduced glucose transport to the brain (Ghajar *et al.*, 1982), a change in plasma concentration of neurotransmitter precursors (Wurtman *et al.*, 1981) or a direct action of this hormone in the central nervous system (Van Houten and Posner, 1981). Indeed, Toth and Lajtha (1981) have shown that glutamate and GABA levels decrease following insulin administration but the effect of this peptide on central monoamine levels has not been detailed in this study. McCaleb and Myers (1979) showed that the efflux of dopamine (DA) increased in push pull perfusates of the rat's caudate nucleus when insulin was added to the perfusate. More recently, Cottet-Emard and Peyrin (1982) reported an increase in dopaminergic activity in the striatum and the hypothalamus, reflected by a rise in the tissue level of homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC).

We have recently described a method for chronic withdrawal of CSF that is suitable for the analysis of monoamine metabolites fluctuations (Danguir *et al.*, 1982; Elghozi *et al.*, 1983). In the present study we have investigated the effect of severe insulin-induced hypoglycemia on free DOPAC, HVA and 5-hydroxyindolacetic acid (5-HIAA). These are the most concentrated free metabolites of DA and 5-HT in rat CSF. We have also determined glucose levels both in blood and in CSF before and after insulin administration in order to provide further indication on the probable relationship between brain glucose fluctuations and monoamine neuronal function.

EXPERIMENTAL PROCEDURES

Animals and housing

Eleven Wistar male rats, weighing 300–350 g were used in this study. They were housed in individual cages in a temperature regulated (24 ± 1°C) room illuminated from 08:00 to 20:00. The cages were Plexiglas cylinders open at

the top to allow chronic CSF removal in freely moving animals. Standard food (Extralabo M25, Pietrement) and water were available *ad libitum* except when mentioned.

Surgery

Rats were anesthetized with pentobarbital sodium (Nembutal, 40 mg/kg). Using a stereotaxic apparatus, a stainless steel cannula with its mandrel was placed in the anterior part of the third ventricle (coordinates A = 6.0, L = 0, V = dura − 6.8). During the same surgical session, six rats were also implanted with an intravenous (auricular) catheter, as described elsewhere (Nicolaïdis *et al.*, 1974). The cranial end of the catheter was cemented besides the implanted cannula.

Insulin-induced hypoglycemia and sampling procedures

All the experiments were performed in the rats home cages. Animals were allowed at least one week for recovery following surgery. CSF was removed continuously from the third ventricle at a constant rate of 1 μl/min with the use of a double barrelled cannula as previously detailed (Danguir *et al.*, 1982). A needle shorter than the implanted guide was screwed on the latter. The inner needle was then connected to a roller pump (Gilson Minipuls 2) through a watertight swivel and PE tubing. CSF samples were collected on ice in Eppendorf tubes every 15 min.

Rats which were implanted with intracardiac catheters were connected through a double lumen swivel to the roller pump for simultaneous blood and CSF samplings.

Following a period of 90 min of control CSF and blood samplings, insulin (6 IU/Kg in 0.5 ml of saline, Novo Actrapid) or saline (0.5 ml) were injected ip while food was removed until the end of the experiment. In one experiment, food was not removed and in another one glucose (1.5 g in 3 ml solution) was administered ip 45 min after insulin administration. Finally, in one experiment, glucose was administered only when the rat reached the coma state as a result of the insulin administration.

Dopamine and serotonin metabolites determination

Liquid chromatography with electrochemical detection was used for the direct assay of free DOPAC, 5-HIAA and HVA in 5 μl samples, as previously described (Elghozi *et al.*, 1983). The running buffer was a 0.1 M citric monohydrogen phosphate buffer (3:2, v/v) containing 20% methanol at pH 3.3. The liquid chromatographic system consisted of a 6000 A solvent delivery system, a U6K injector and a μBondapak C18 column from Waters (Milford, U.S.A.). The eluting flow rate was adjusted to 1.0 ml/min. The electrochemical detection system consisted of a thin layer TL-4 flow cell from BAS (West Lafayette, U.S.A.) and a PRG-DEL electronic controller from Tacussel (Lyon, France). The carbon paste working electrode was set to a potential of +800 mV against a silver-silver chloride reference electrode filled with 3 M NaCl solution. The chromatograms were displayed on a Servotrace recorder type PE from Sefram (Paris, France). Fresh standard mixtures were chromatographed the same day as CSF samples. The monoamine metabolites were identified and quantified by comparing their retention times and peak heights to those of the standards. Retention times for DOPAC, 5-HIAA and HVA were 5.5, 7.3 and 8.7 min respectively.

Glucose assay

Glucose levels both in CSF and blood were measured using the glucose oxidase method (glucose analyser YSI model 23A, U.S.A.). Since glucose level determinations were performed immediately after sampling, heparine was not added to blood samples.

Behavioral analysis

Rats were categorized as stuporous or comatose according to their orientation to sound stimuli. Compared to normal awake rats, stuporous rats showed a diminished orienting response and comatose rats had no orienting response.

RESULTS

Figure 1 represents the effects of insulin administration dopamine (DOPAC and HVA) and serotonin (5-HIAA) metabolites in CSF of rats which had no access to food during the 4 h experiment. The control period was characterized by a stable level of these three compounds. A progressive increase of the 3 metabolites starting 75 min and reaching its highest values 150 min after insulin administration was then observed. This increase was significant ($P < 0.02$, paired t-test) administration on and after the 105th min following insulin (Fig. 1). The maximum concentrations were respectively 133, 180 and 147% of the basal control levels for 5-HIAA, DOPAC and HVA. Stupor was observed in all animals 30 min after insulin administration when DA and 5-HT metabolites in CSF started increasing (Fig. 1). The coma

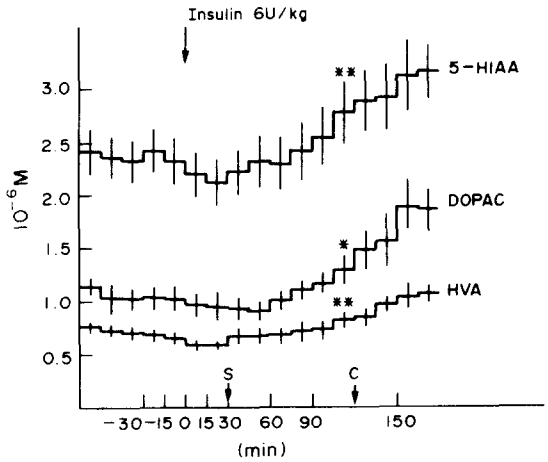

Fig. 1. DOPAC, HVA and 5-HIAA levels before and after the ip administration of insulin (6 IU/Kg). Results are expressed as the means ± SEM ($n = 8$). Abscissa time. Insulin was injected at 0 time. Rats had no access to food during the 4 h experiment. Statistical significance was reached 105 min after insulin: $P < 0.02$, $P < 0.01$ (paired t-test by comparing each time period level with the pre-insulin (−15 min) level. Stupor (S) and Coma (C) states were observed 30 min and 120 min respectively after insulin.

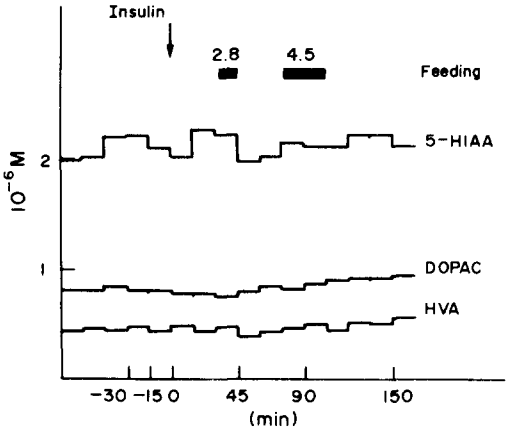

Fig. 2. Dopamine (DOPA and HVA) and serotonin (5-HIAA) metabolites levels in CSF before and after insulin (6 IU/Kg) administration in one rat which had free access to food. Two meals (2.8 and 4.5 g) followed the insulin injection (at time 0). No behavioral alterations were observed. Similar results were obtained for 2 other rats.

state was observed when the levels of monoamine metabolites reached their highest values, i.e. 150 min after insulin injection (Fig. 1). No changes in acidic metabolites levels were observed when food was accessible (Fig. 2), when glucose (5g/kg ip) was administered 45 min after insulin (Fig. 3) or when saline was injected instead of insulin (Fig. 4). However, when a similar glucose injection was given once the rat had entered coma, it had no effect on monoamine metabolites which remained high for DOPAC and HVA and even continued increasing for 5-HIAA (Fig. 5).

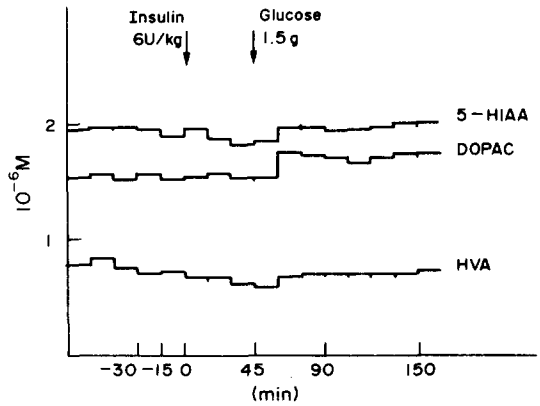

Fig. 3. DOPAC, HVA and 5-HIAA levels in CSF before and after insulin administration which was followed 45 min later by an ip injection of glucose (5g/Kg) in one of the 3 rats used in this experiment. Food was removed during the 4 h experiment.

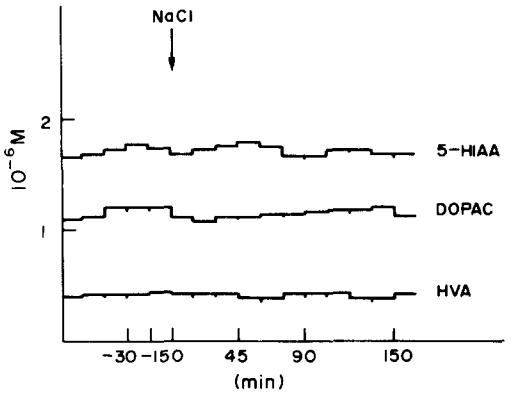

Fig. 4. Effect of ip saline administration (0.5 ml) on DOPAC, HVA and 5-HIAA levels in CSF. Notice the stable levels of the 3 metabolites during the entire experiment. No food was available. Similar results were obtained in 4 other rats.

Interestingly, the highest levels of monoamine metabolites were observed when glucose levels in CSF reached their lowest values (0.12 g/l vs 0.42 g/l before insulin) (Fig. 6). Following the administration of insulin, the expected fall in CSF glucose occurred 45 min after that observed for blood glucose (Fig. 7). A similar time-lag between blood and CSF glucose variations was also observed after the injection of glucose (Fig. 7).

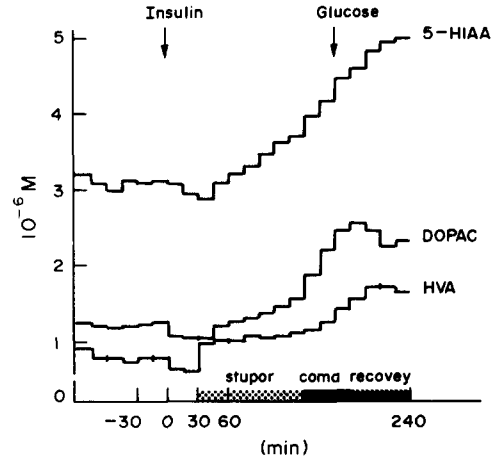

Fig. 5. Effect of glucose (5 g/Kg) injection during the coma state induced by an insulin administration on DOPAC, HVA and 5-HIAA levels in CSF. Results from one rat are represented. Similar results were observed in 3 other rats. Notice that DOPAC remained high but stable whereas 5-HIAA continued increasing despite the behavioral recovery (locomotion, grooming, orientation to sound stimuli etc) which followed the administration of glucose. No food was available. Complementary data are shown on Fig. 7.

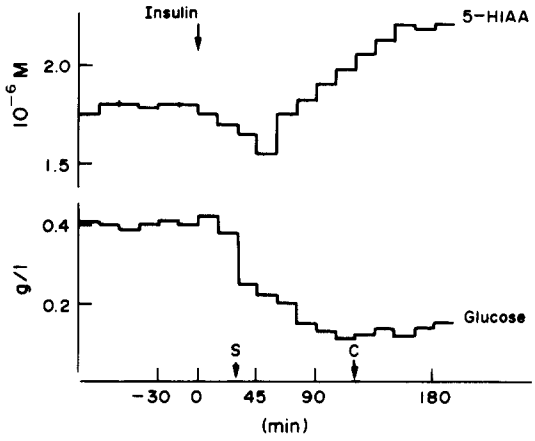

Fig. 6. Simultaneous variations of glucose and 5-HIAA levels in one of the 8 rats (see Fig. 1) which received acute insulin administration. Notice that stupor (S) coincides with the abrupt fall of CSF glucose (30 min after insulin). Coma (C) state was reached when glucose in CSF was the lowest (0.12 g/l). Food was not available during the 4 h experiment.

DISCUSSION

The main finding of this study is the simultaneous increase of DA and 5-HT metabolites in the CSF during severe insulin-induced hypoglycemia in rats which had no access to food during the 4 h experiment. The elevation of the metabolites occurred while animals were stuporous. This could reflect an elevated brain monoamine release in structures located near the ventricles. Indeed, McCaleb and Myers (1973) found, in push-pull perfusion studies, an immediate rise in the efflux of striatal DA when insulin was added to the perfusate; conversely an infusion of glucose decreased DA release. Similarly, Cottet-Emard and Peyrin (1982) reported insulin-induced (injected ip) increase in DA metabolites in the striatum and the hypothalamus of the rat.

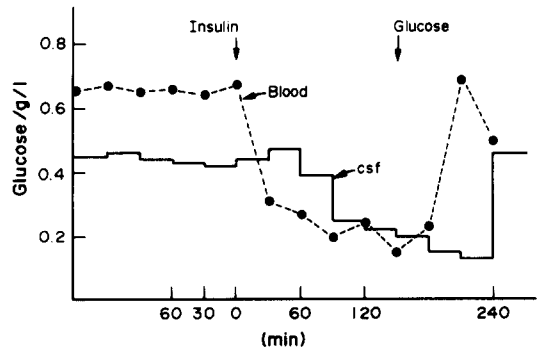

Fig. 7. Simultaneous changes in blood and CSF glucose following insulin administration and glucose injection during the coma state of rats shown in Fig. 5. Glucose levels in CSF are represented by histograms as each determination was performed on a 30 min collected samples. Blood glucose levels were determined on acute withdrawn samples.

Insulin caused an abrupt decrease in CSF glucose, approximately 45 min after the injection, just when the monoamine metabolites levels started increasing and while rats are in stupor state. This finding would suspect a primary role for glucose in the metabolism of brain amines. This alteration in CSF glucose level could be associated with similar fluctuations in brain extracellular fluid glucose concentrations since mechanisms regulating the glucose concentration in the CSF seem to be indistinguishable from those operating at the blood–brain interface (Cutler, 1980). Such a role of brain glucose in monoamine neuronal activity is strengthened by the absence of effect of insulin on CSF monoamine metabolites levels when rats were allowed to compensate their hypoglycemia by food ingestion or when blood and CSF glucose levels were preserved by glucose administration. However, a late glucose administration performed when rats were already stuporous or comatose did not affect DA and 5-HT metabolites levels which remained high. Similarly, Ghajar *et al.* (1982) reported that neurological function was not restored after prolonged hypoglycemic stupor. In our experiments, similar irreversible neurological damage could be responsible for the absence of restoration of normal acidic metabolites levels when glucose was given once the rat was in coma. Whether this change could be reversible or not, the mechanisms triggering an activation of DA and 5-HT metabolism should be discussed. Besides the probably prominent role of brain glucose in the regulation of monoamine metabolism, demonstrated by the effect of glucose injection and exemplified by the negative correlation between CSF glucose and metabolites levels, two other mechanisms could be implicated. Firstly, insulin is known to influence the brain amounts of precursors available to the neurons. Indeed, insulin administration or its endogenous secretion after carbohydrate consumption were shown to raise the ratio of the plasma tryptophan to the sum of the other, competing, large neutral aminoacid concentrations in plasma (Wurtman *et al.*, 1981). This results in elevated brain tryptophan levels thus increasing the saturation of the enzyme (tryptophan hydroxylase) that controls the rate at which neurons synthesize 5-HT. Secondly, insulin can reach the CSF and then, presumably, the brain tissue after its administration into the peripheral circulation (Margolis and Altszuler, 1967;

Woods and Porte, 1977). The peripherally administered insulin in this experiment could reach the brain and mimick the possible, yet unknown, effect of brain insulin on monoamine metabolites either by lowering glucose levels or by acting directly on neurons. The absence of effect of the injection of glucose on the increased level of DA and 5-HT metabolites during the insulin-induced coma state would favor this possible direct effect of insulin on brain cells. Similarly, the increased duration of sleep following intracerebroventricular infusion of insulin recently found in the rat (Danguir, Submitted) would strengthen the idea of a direct involvement of this hormone in neuronal activity. The recent discovery of insulin receptors within the rat brain (Havrankova *et al.*, 1981) could corroborate such an interpretation.

REFERENCES

Cillufo, J. M., Anderson, R. E., Mechenfelder, J. D. and Sundt, T. M. (1982). Cerebral blood flow, brain pH, and oxidative metabolism in the cat during severe insulin-induced hypoglycemia. *J. Cereb. Blood Flow Metab.* **2,** 337–346.

Cottet-Emard, J. M. and Peyrin, L. (1982). Conjugated HVA increase in rat urine after insulin-induced hypoglycemia: Involvement of central dopaminergic structures but not of adrenal medulla. *J. Neural Trans.* **55,** 121–138.

Cutler, R. W. P. (1980). Neurochemical aspects of blood-brain-cerebrospinal barriers. In: *Neurobiology of the Cerebrospinal Fluid* (Wood, J. H., ed.) pp. 41–51. Plenum Press, London.

Danguir, J., Le Quan-Bui, K. H., Elghozi, J. L., Devynck, M. A. and Nicolaidis, S. (1982). LCEC monitoring of 5-hydroxyindolic compounds in the cerebrospinal fluid of the rat related to sleep and feeding. *Brain Res. Bull.* **8,** 293–297.

Danguir, J. (1983). The hypnogenic properties of insulin. *Nature, Lond.* Submitted.

Elghozi, J. L., Mignot, E. and Le Quan-Bui, K. H. (1983). Probenecid sensitive pathway of elimination of dopamine and serotonin metabolites in CSF of the rat. *J. Neural Trans.* In press.

Ferrendelli, J. A. and Chang, M. M. (1973). Brain metabolism during hypoglycemia. Effect of insulin on regional central nervous system glucose and energy reserves in mice. *Arch. Neurol.* **28,** 171–177.

Ghajar, J. B. G., Plum, F. and Duffy, T. E. (1982). Cerebral oxidative metabolism and blood flow during acute hypoglycemia and recovery in unanesthetized rats. *J. Neurochem.* **38,** 397–709.

Havrankova, J., Brownstein, M. and Roth, J. (1981). Insulin and insulin receptors in rodent brain. *Diabetologia* **20,** 268–273.

Kety, S. S., Woodford, R. B., Harmel, M. H., Freyhan, F. A., Appel, K. E. and Schmidt, C. F. (1948). Cerebral blood flow and metabolism in schizophrenia. The effects of barbiturate semi-narcosis, insulin coma and electroshock. *Am. J. Psychiat.* **104,** 765–770.

Lewis, L. D., Ljunggren, B., Norberg, K. and Siesjö, B. K. (1974). Changes in carbohydrate substrates, amino acids and ammonia in the brain during insulin-induced hypoglycemia. *J. Neurochem.* **23,** 659–671.

Margolis, R. U. and Altszuler N. (1967). Insulin in the cerebrospinal fluid. *Nature* **215,** 1375–1376.

McCaleb, M. L. and Myers, R. D. (1979). Striatal dopamine release is altered by glucose and insulin during push-pull perfusion of the rat's caudate nucleus. *Brain Res. Bull.* **4,** 651–656.

Nicolaidis, S., Rowland, N., Meile, M. J., Marfaing-Jallat, P. and Pesez, A. (1974). A flexible technique for long term infusions in unrestrained rats. *Pharmac. biochem. Behav.* **2,** 131–136.

Toth, J. and Lajtha, A. (1981). Drug-induced changes in the composition of the cerebral free amino acid pool. *Neurochem. Res.* **6,** 3–12.

Van Houten, M. and Posner, B. I. (1981) Cellular basis for direct insulin action in the central nervous system. *Diabetologia* **20,** 255–267.

Woods, S. C. and Porte, D. Jr. (1977). Relationship between plasma and cerebrospinal fluid insulin levels of dogs. *Am. J. Physiol.* **2,** E331–E334.

Wurtman R. J., Hefti, F. and Melamed, E. (1981). Precursor control of neurotransmitter synthesis. *Pharmac. Rev.* **32,** 315–335.