cubically packed system represent the maximum possible values that can be achieved with a model of monosize cubes.

It appears, therefore, that Eq. 9 will satisfactorily estimate the tensile strengths of the moist, inherently noncohesive sodium chloride fractions.

CONCLUSION

Two nonporous bulk solids of similar particle size but different coherences were shown to exhibit, at similar porosities, different changes in tensile strength with increasing moisture content. The more cohesive material (potassium chloride) showed similar changes to the fine sodium chloride fractions studied previously (1), whereas the less cohesive one (glass) resembled the coarse sodium chloride fractions.

Mechanisms for the tensile strength changes are suggested, and the type of change obtained appears to be dependent on the magnitude of the inherent coherence of the dry material and not necessarily on its particle size.

The cohesive calcium phosphate differed from the other cohesive bulk solids studied, however, in that its tensile strength at a fixed porosity was independent of the moisture content over a wide range. This finding was attributed either to the porous nature of the particles preventing location of the moisture at the particle surface or to a fortuitous balance between the components of tensile strength which increase with moisture content and those that decrease.

REFERENCES

(1) T. Eaves and T. M. Jones, *J. Pharm. Sci.*, **61**, 256(1972).

(2) T. Eaves and T. M. Jones, *J. Pharm. Pharmacol.*, **22**, 594 (1970).

(3) T. Eaves and T. M. Jones, *Rheol. Acta*, **10**, 127(1971).

(4) R. Farley and F. H. H. Valentin, *Powder Technol.*, **1**, 344 (1967/68).

(5) D. M. Newitt and J. M. Conway-Jones, *Trans. Inst. Chem. Eng.*, **36**, 422(1958).

(6) E. Bock, *Adhesion*, **7**, 289(1965).

(7) R. Aoki and H. Tsunakawa, *J. Soc. Mater. Sci. Japan*, **18**, 497(1969).

(8) J. Craik and B. F. Miller, *J. Pharm. Pharmacol.*, **10**, 136T (1958).

(9) V. E. Glushkov, N. I. Karnaushenko, and P. V. Platonov, Paper B.4.3., 3rd Congress CHISA, Marianbad, Czechoslovakia, Sept. 1969.

(10) H. Rumpf, in "Agglomeration," W. A. Knepper, Ed., Interscience, London, England, 1962.

(11) H. C. Hamaker, *Physica*, **4**, 1027(1937).

(12) W. B. Pietsch and H. Rumpf, *Chem.-Ing.-Tech. Z.*, **39**, 885 (1967).

(13) W. B. Pietsch, *Staub*, **27**, 24(1967).

(14) W. B. Pietsch, E. Hoffmann, and H. Rumpf, *Ind. Eng. Chem. Prod. Res. Develop.*, **8**, 58(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 15, 1971, from the *Department of Pharmacy, University of Nottingham, Nottingham NG7 2RD, England.*

Accepted for publication October 19, 1971.

The authors thank The Boots Co. Ltd. for the tensile testing apparatus and the Agnes Borrowman Trust for a grant to T. Eaves.

* Present address: Sandoz Products Ltd., Horsforth, Leeds, England.

▲ To whom inquiries should be directed.

Effect of Lysergide and Nimergoline on Glucose Metabolism Investigated on the Dog Brain Isolated *In Situ*

G. BENZI▲, M. De BERNARDI, L. MANZO, A. FERRARA, P. PANCERI, E. ARRIGONI, and F. BERTÉ

Abstract □ The glucose uptake and lactic and pyruvic acid formation were investigated in the dog brain isolated *in situ* during: (*a*) control conditions, (*b*) hypoxia by suppression of blood oxygenation, and (*c*) recovery of oxygenation and intracarotid treatment with saline solution or lysergide (5×10^{-7} *M*) or nimergoline (5×10^{-5} *M*) at the rate of 0.5 ml./min. for 30 min. The hypoxia induced at first an increase and subsequently a decrease in brain glucose uptake, with an increase in both lactic and pyruvic acid formation. The subsequent *recovery of blood oxygenation*, with intracarotid perfusion with saline solution, induced only a partial recovery of glucose metabolism supported by the increase of the depressed glucose uptake and the decrease of the enhanced lactic and pyruvic acid formation. No significant difference could be demonstrated *versus* the intracarotid perfusion with lysergide. The intracarotid perfusion with nimergoline induced both an increase in cerebral glucose uptake and a decrease in pyruvate formation, with statistical difference with respect to the change induced by saline solution perfusion; the lactic acid formation remained in the same order of magnitude induced by saline solution perfusion.

Keyphrases □ Glucose metabolism, isolated dog brain *in situ*—effect of lysergide and nimergoline □ Lysergide—effect on glucose metabolism in isolated dog brain *in situ* □ Nimergoline—effect on glucose metabolism in isolated dog brain *in situ* □ Lactic acid formation, isolated dog brain *in situ*—effect of lysergide and nimergoline □ Pyruvic acid formation, isolated dog brain *in situ*—effect of lysergide and nimergoline

The predominant source of energy for the brain comes from carbohydrate metabolism, the glucose being the major, if not exclusive, energy-yielding substance removed from the blood to the brain. In fact, according to Kety (1), the respiratory quotient of the brain is near one. On the other hand, according to Bloom (2) and Sacks (3), brain slices appear to catabolize glucose practically up to the end *via* the Embden–Meyerhof

pathway. Nevertheless, using a semiisolated calf brain preparation, Moss (4) maintained that the glucose ultimately passes for the most part, if not entirely, through the hexose monophosphate shunt.

In any case, glucose and its metabolites, particularly lactic acid and pyruvic acid, may play a primary role in maintaining normal brain metabolism and function. According to Geiger *et al.* (5), in the intact animal it is practically impossible to single out those blood components that are essential to the maintenance of normal brain metabolism and function. However, perfusion methods in the isolated brain are suitable for this purpose, because the composition of the perfusing blood, the blood flow rate, and the blood oxygenation may be selected at will.

A cat brain perfusion method *in vivo* was described by Geiger and Magnes (6) and, subsequently, a number of changes were made (5, 7, 8). White *et al.* (9) reported an anatomic isolation of the monkey brain which was capable of maintaining electrocortical activity for several hours when the cerebral circulation was provided by a compatible donor monkey. Gilboe *et al.* (10) described the isolation and mechanical perfusion of a viable dog head and subsequently (11) described the neurogenic and vascular isolation and maintenance of the living dog brain. Benzi *et al.* (12) investigated the cerebral drug-metabolizing activity by studying the demethylation, the acetylation, and the glucurono conjugation by the *in situ* isolated dog and monkey brain. Subsequently, the research (13) was extended to the newborn dog, and the *in situ* isolated brain showed a drug-metabolizing activity related to age. Benzi *et al.* (14) found that intracarotid perfusion of ephedrine and nimergoline, but not lysergide, induces changes in the drug-metabolizing activity of the dog brain isolated *in situ*. This behavior suggests the ability of the quoted substances to act also on the cerebral metabolism. To test this hypothesis, glucose uptake and lactic and pyruvic acid formation were investigated in the isolated dog brain *in situ* before and after the induction of a transient condition of local hypoxia and subsequent pharmacological treatment.

METHOD

The experiments were performed in the dog using the technique of the isolated perfused brain *in situ*.

Animals and Anesthesia—The experiments were carried out on 18 beagle dogs (11.3–15.2 kg. body weight), which were preanesthetized with urethan (0.4 g./kg. i.p.). Anesthesia was induced and maintained by nitrous oxide, cyclopropane, or ethyl ether in closed circuit. Electrical activity of the brain as portrayed by ECG was used to determine the degree of anesthesia (15).

The animals were artificially ventilated after tracheal intubation with a Warne tube following succinylcholine chloride (1 mg./kg. i.v.) administration. Anesthesia was stopped 30–40 min. before connecting the isolated brain to the pump-oxygenator system. The ECG pattern restoration indicated the time allowed for a "blow-off" of anesthetic gases before beginning the experiment on cerebral glucose metabolism.

Operative Procedure—The operative procedure (12) consisted mainly of the isolation of the external jugular vein and the common carotid arteries, with ligature of all their branches except the internal carotid arteries and the right superior thyroidea artery. In addition, the vertebral vessels were ligated before their entrance into the transverse foramen of C2 or C3. The numerous muscular branches arising from the vertebral vessels, the anastomosis between vertebral and carotid arteries, the anastomosis between vertebral and jugular veins, the internal jugular veins, the vascular branches of the neck, the vessels running under the carotid arteries and vagus nerves, and the zygomatic, maxillary, auricular, and supraorbital vessels were all occluded by ligature or compression. The occlusions of the sinus columnae vertebralis and of the anterior spinal artery were made by opening the rachis in C2, ligating the spinal artery, and compressing the venous vessels around the spinal cord.

The isolated right superior thyroidea artery was cannulated by a polystan tube and connected to a perfusor apparatus (Palmer). Both of the isolated jugular veins were ligated, cannulated, and connected to the venous reservoir of the pump-oxygenator system (through the gravitational flow). Both of the isolated carotid arteries were also cannulated and connected to the pump-oxygenator system. Arterial blood pressure was measured from a cannula inserted into a femoral artery. During the experiments, the frontooccipital electroencephalogram as well as both systemic and cerebral pressure were recorded on a 12-channel polygraph.

Brain Pump-Oxygenator System—The brain perfusion apparatus employed consisted of a venous reservoir, an oxygenator with a gasmeter, a roller-type pump with a flowmeter, two blood filters (glass wool and Dacron wool), an apparatus to eliminate blood foam, a perfusion pressure regulator with a manometer, and a blood exchanger with a telethermometer. Before the extracorporeal perfusion, the pump-oxygenator system was filled with 500 ml. of heparinized compatible blood. The blood was obtained 20 min. prior to use to prevent the accumulation of lactic acid, which would subsequently require a considerable degree of neutralization.

Before the perfusion, the blood was filtered through glass wool and polyester staple and adjusted to pH 7.35 using 1 *M* sodium bicarbonate. The priming blood, which was fully oxygenated and warmed, was circulated through the pump-oxygenator system. A flow of a $O_2 + CO_2$ mixture (95:5), maintained at the rate of 5–8 l./min., was passed into the oxygenator during the extracorporeal brain perfusion. The blood flow rate was kept between 4 and 6 ml./min./kg., the pressure being equal to the initial systemic pressure of the animal. The time of brain perfusion was limited to 60–90 min. and was related to the presence of a considerable cerebral electric activity.

The leakage of perfusate, if any, into the systemic circulation was evaluated at the end of the experiment by adding either a dye or a radiopaque substance to the blood of the extracorporeal circuit.

The data reported in the *Results* section concern only the experiments ($n = 18$) without valuable leakage of perfusate. In other experiments ($n = 7$), leakage occurred, and the findings on cerebral glucose metabolism were not considered because of the interference of the body metabolic pool.

Cerebral Glucose Metabolism—Glucose uptake and lactic or pyruvic acid formation were calculated in mg./100 g. brain/min. from simultaneously drawn arterial and venous blood samples. In fact, according to Gilboe *et al.* (16), the rate of lactic acid formation by the erythrocytes in the perfusion system is sufficiently high to cause interference with the precise determination of the lactic acid released by the brain. Such interference is substantially decreased by drawing arterial and venous samples simultaneously and precipitating the blood protein immediately. By continuous addition of glucose to the venous reservoir, the glucose concentration in blood was maintained at a constant level.

Study Program—The glucose metabolism was investigated during three periods: (*a*) during the 15-min. period of control conditions, (*b*) during and at the end of the 15-min. period of hypoxia induced by stopping the blood oxygenation, and (*c*) at the end of the 30-min. period of both the blood oxygenation recovery and the saline solution or drug treatment. The drug perfusion was made through the superior thyroidea artery (at the rate of 0.5 ml./min. for 30 min.) with: (*a*) saline solution; (*b*) *N,N*-diethyl-D-lysergamide (lysergide), 5×10^{-7} *M;* and (*c*) 1,6-dimethyl-8β-(5-bromonicotinoyloxymethyl)-10α-metoxyergoline tartrate (nimergoline or nicergoline), 5×10^{-6} *M*.

Lysergide and nimergoline concentrations were based on the doses employed in clinical application to man (0.03–0.04 to 0.2–0.3 mg. of lysergide and 2–4 to 10–20 mg. of nimergoline).

RESULTS AND DISCUSSION

Glucose Metabolism during Control Conditions—As indicated in Table I, the glucose uptake (evaluated in mg./100 g. brain/min.)

Table I—Glucose Uptake and Lactic and Pyruvic Acid Formation (mg./100 g. Brain/min.) in the Isolated Dog Brain *In Situ*, in Control Condition and in Hypoxia, with Subsequent Recovery of Blood Oxygenation with Intracarotid Treatment with Saline Solution or Lysergide (5×10^{-7} *M*) or Nimergoline (5×10^{-6} *M*), at the Rate of 0.5 ml./min. for 30 min.[a]

	n[b]	Glucose Uptake	Lactic Acid Formation	Pyruvic Acid Formation
During control conditions (a)	18	4.76 ± 1.23	0.35 ± 0.02	0.14 ± 0.05
At end of hypoxia (b)[c]	18	2.28 ± 0.18	0.79 ± 0.05	0.91 ± 0.10
At end of 30-min. period of treatment (c)[d]				
$O_2 + CO_2$ + saline (c_1)	6	3.23 ± 0.50	0.49 ± 0.07	0.52 ± 0.09
$O_2 + CO_2$ + lysergide (c_2)	5	3.38 ± 0.54	0.44 ± 0.07	0.51 ± 0.10
$O_2 + CO_2$ + nimergoline (c_3)	7	4.07 ± 0.36	0.54 ± 0.08	0.37 ± 0.07

[a] The statistical evaluation of the difference between (↔) the various experimental conditions was obtained by Student's *t* test. These calculated levels of significance are:

Glucose Uptake: a ↔ b = $p < 0.01$

$c_1 \leftrightarrow a = p < 0.01$	$c_1 \leftrightarrow b = p < 0.01$	$c_1 \leftrightarrow c_2 = p > 0.05$
$c_2 \leftrightarrow a = p < 0.01$	$c_2 \leftrightarrow b = p < 0.01$	$c_1 \leftrightarrow c_3 = p < 0.05$
$c_3 \leftrightarrow a = p < 0.05$	$c_3 \leftrightarrow b = p < 0.01$	$c_2 \leftrightarrow c_3 = p < 0.05$

Lactic Acid Formation: a ↔ b = $p < 0.01$

$c_1 \leftrightarrow a = p < 0.05$	$c_1 \leftrightarrow b = p < 0.05$	$c_1 \leftrightarrow c_2 = p > 0.05$
$c_2 \leftrightarrow a = p < 0.05$	$c_2 \leftrightarrow b = p < 0.05$	$c_1 \leftrightarrow c_3 = p > 0.05$
$c_3 \leftrightarrow a = p < 0.05$	$c_3 \leftrightarrow b = p < 0.05$	$c_2 \leftrightarrow c_3 = p > 0.05$

Pyruvic Acid Formation: a ↔ b = $p < 0.01$

$c_1 \leftrightarrow a = p < 0.01$	$c_1 \leftrightarrow b = p < 0.01$	$c_1 \leftrightarrow c_2 = p > 0.05$
$c_2 \leftrightarrow a = p < 0.01$	$c_2 \leftrightarrow b = p < 0.01$	$c_1 \leftrightarrow c_3 = p < 0.05$
$c_3 \leftrightarrow a = p < 0.01$	$c_3 \leftrightarrow b = p < 0.01$	$c_2 \leftrightarrow c_3 = p < 0.05$

[b] n = number of isolated dog brain preparations. [c] Suppression of the $O_2 + CO_2$ flow into the oxygenator. [d] Recovery of $O_2 + CO_2$ flow into the oxygenator and perfusion through the thyroidea superior artery (at the rate of 0.5 ml./min. for 30 min.) with saline solution or lysergide (5×10^{-7} *M*) or nimergoline (5×10^{-6} *M*).

was 4.76 ± 1.23, in agreement with the data of Gilboe *et al.* (16) in two groups of isolated dog brains (4.90 ± 1.00 and 3.65 ± 0.73), of Geiger and Magnes (6) in perfused cat brain (5.87 ± 0.45), and of Gottstein *et al.* (17) (5.30 ± 0.96) and of Kety (1) on brain of human subjects (5 mg./100 g. brain/min.).

The lactic acid formation (in mg./100 g. brain/min.) was 0.35 ± 0.02, in agreement with the data reported by Gilboe *et al.* (16) in isolated dog brains (0.30 and 0.43) and by Gottstein *et al.* (17) in normal human subjects (0.42). As indicated in Fig. 1, the 7.35% of the glucose taken up by the brain was transformed into lactic acid. According to Gilboe *et al.* (16), the lactic acid formation accounted for 6.1% of the glucose uptake in the Dacron wool-filtered perfusion blood (as in the present experimental conditions) and 11.7% in screen-filtered perfusion blood. Himwich and Himwich (18) observed 11.8% conversion of glucose to lactic acid; but on the basis of reports of cerebral glucose and total brain oxygen utilization (1), Gilboe *et al.* (16) considered it unlikely that the brain normally converts more than 5–6% of the glucose to lactic acid. In any case, even in normal subjects, some cerebral anaerobic metabolism occurs (19, 20).

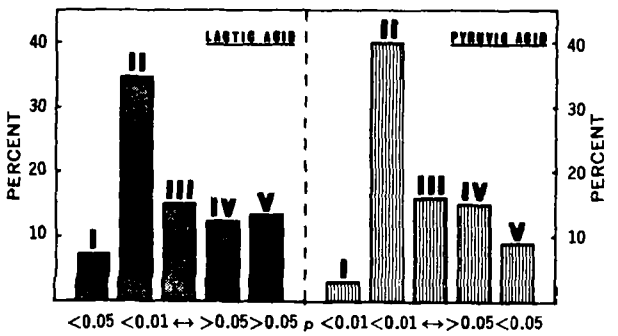

Figure 1—*Formation of lactic acid and pyruvic acid represented as percentage (ordinate) of the glucose uptake in isolated* in situ *dog brain during the control condition (I), the hypoxia (II), and the recovery of blood oxygenation with intracarotid perfusion with saline solution (III) or lysergide (5×10^{-7} M) (IV) or nimergoline (5×10^{-6} M) (V), at the rate of 0.5 ml./min. for 30 min. The numbers below the figure indicate the probability levels of the difference between the data of III in respect to other conditions.*

The pyruvic acid formation (in mg./100 g. brain/min.) was 0.14 ± 0.05, in agreement with the data of Gilboe *et al.* (16) in one group of isolated dog brains (0.13 ± 0.05) and of Gottstein *et al.* (17) in normal human subjects (0.055 ± 0.054). As indicated in Fig. 1, only 2.94% of glucose taken up by the brain was found as pyruvic acid.

Glucose Metabolism during Hypoxia—The hypoxia resulted at first in an increase of the cerebral glucose consumption (about 32%); but at the end of the hypoxia period (PaO_2 = 19.7 ± 0.85 mm. Hg), a significant decrease in brain glucose uptake occurred (Table I). The decrease of glucose uptake accounted for about 52% of the control values (Fig. 2). On the other hand, a significant increase of both lactic acid and pyruvic acid formation was observed (Table I and Fig. 2).

At the end of the present condition of experimental hypoxia following the stopping of blood oxygenation, it was calculated that lactic acid formation accounted for 34.6% and pyruvic acid formation accounted for 39.9% of glucose taken up by the isolated brain (Fig. 1). This shows an essential tendency toward an anaerobic metabolism of glucose with accumulation of pyruvic acid caused by the block of the pathways of normal glucose utilization. On the other hand, Meyer *et al.* (21) found that cerebral lactate production can be increased without any change in cerebral pyruvate production. This observation tends to confirm the view that the increase in lactate production is not due to a total increase in cerebral glycolysis (22–24).

Glucose Metabolism after Recovery of Blood Oxygenation and Pharmacological Treatment—*Treatment by Saline Solution*—The recovery of $O_2 + CO_2$ flow into the oxygenator and the perfusion through the superior thyroidea artery with saline solution for 30 min. (Table I and Fig. 2) increased the brain glucose uptake depressed by hypoxia and decreased the lactic acid and the pyruvic acid formation. At the end of the 30-min. period of blood oxygenation

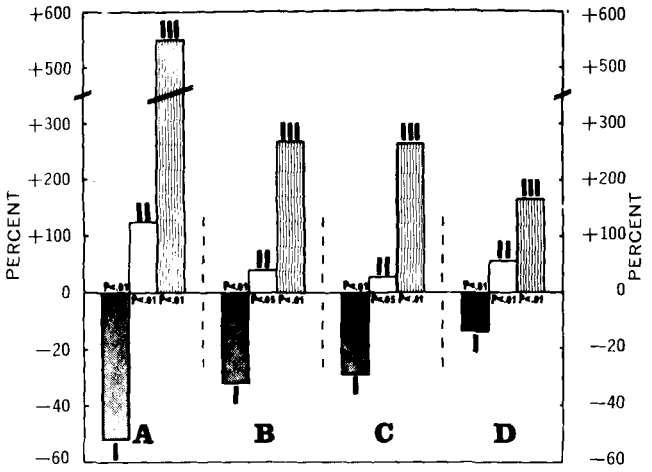

Figure 2—*Change of glucose uptake (I) and lactic (II) and pyruvic (III) acid formation (represented in ordinate) as a mean percent variation (with probability levels) from the control values, during the hypoxia (A) and the recovery of blood oxygenation with intracarotid perfusion with saline solution (B) or lysergide (5×10^{-7} M) (C) or nimergoline (5×10^{-5} M) (D), at the rate of 0.5 ml./min. for 30 min.*

recovery, the lactic acid formation accounted for 15.2% and the pyruvic acid formation accounted for 16.1% of the glucose taken up by the isolated brain (Fig. 1). In any case, the recovery of blood oxygenation induced only a partial recovery of glucose metabolism as evaluated during the control conditions. This behavior indicates that (*a*) the experimental cerebral hypoxia in the present research results in anaerobic glycolysis which remains reversible, and (*b*) the permanence of a higher than normal tendency toward anaerobic metabolism of glucose, also after a 30-min. period of blood oxygenation recovery, shows the presence of an unphysiologic process.

Treatment by Lysergide—The recovery of $O_2 + CO_2$ flow into the oxygenator and the perfusion through the superior thyroidea artery with lysergide (5×10^{-7} *M*, at the rate of 0.5 ml./min.) for 30 min. induced a change in glucose metabolism of the same extent as that following the treatment with saline solution. In fact, as indicated in Table I, the brain glucose uptake depressed by hypoxia was increased without any statistical difference *versus* the change induced by saline solution. The lactic acid and pyruvic acid formation, enhanced by hypoxia, was decreased without any statistical difference *versus* the change induced by saline solution perfusion.

The lactic acid formation accounted for 13.2% and pyruvic acid formation accounted for 15.1% of the glucose taken up by the dog isolated brain (Fig. 1).

Treatment by Nimergoline—The recovery of $O_2 + CO_2$ flow into the oxygenator and the perfusion through the superior thyroidea artery with nimergoline (5×10^{-5} *M*, at the rate of 0.5 ml./min.) for 30 min. induced an increase of glucose uptake depressed by hypoxia with statistical difference *versus* the change induced by saline solution. The lactic acid formation, enhanced by hypoxia, was decreased without any statistical difference *versus* the change induced by saline solution perfusion. The pyruvic acid formation, enhanced by hypoxia, was decreased with statistical difference *versus* the change induced by saline solution. The lactic acid formation accounted for 13.2% and pyruvic acid production accounted for 9.1% of the glucose taken up by the dog isolated brain (Fig. 1).

Therefore, nimergoline can improve the cerebral glucose uptake with a significant reduction of the block of the pathways of the normal glucose utilization at the pyruvic acid stage, without significant reduction of the enhanced lactic acid production, *versus* the control perfusion with saline solution.

This behavior indicated: (*a*) the interference of nimergoline upon the oxidative glucose metabolism with a permanence of a partial tendency toward anaerobic metabolism induced by hypoxia; and (*b*) the possibility to dissociate, also from the pharmacological point of view, the cerebral lactate formation from the total cerebral glycolysis, as supported by the quoted physiopathological observations (21–24).

The formulate interference of nimergoline in the pathway for glucose oxidation is supported by other experimental data. According to Moretti and Arcari (25), nimergoline partially restored the mitochondrial phosphorylation efficiency impaired by the administration of high doses of either epinephrine or norepinephrine to rats. Furthermore, in dog brain isolated *in situ*, according to Benzi *et al.* (14), in the absence of hypoxia the drug increased some biotransformations (demethylation, acetylation, and glucurono conjugation) with an increase in cerebral oxygen consumption. On the other hand, in the dog cerebral vascular insufficiency during clamping of the carotid arteries, Cahn[1] showed that nimergoline increased cerebral oxygen utilization, decreased the loss of phosphates, and improved the redox-potential of the lactate-pyruvate system, which could furnish information on energetic conditions in the tissues (26).

CONCLUSION

Previous research on the action of some drugs perfused into the circle of Willis showed that ephedrine and, particularly, nimergoline, but not lysergide, can improve the metabolizing ability (demethylation and acetylation of aminopyrine and glucurono conjugation of oxazepam) of the dog brain isolated *in situ* (14).

In the present research, it was observed that nimergoline, but not lysergide, can interfere with the cerebral metabolism of glucose. This behavior suggests the fitness of specific study on the parallelism between the drug-metabolizing and the metabolic power of the brain to elucidate the role of carbohydrate metabolism as an eventual source of energy for drug-metabolizing activity.

Nimergoline can improve the cerebral glucose uptake and reduce the pyruvic acid formation modified by the experimental hypoxia, without any reduction of the enhanced lactic acid production, *versus* the perfusion with saline solution. Therefore, the drug interferes with the oxidative glucose metabolism, while the enhanced tendency toward anaerobic metabolism persists.

REFERENCES

(1) "Neurochemistry," S. S. Kety, Ed., Charles C Thomas, Springfield, Ill., 1962, p. 119.

(2) B. Bloom, *Proc. Soc. Exp. Biol. Med.*, **88,** 317(1955).

(3) W. Sacks, *J. Appl. Physiol.*, **10,** 37(1957).

(4) G. Moss, *Diabetes*, **13,** 585(1964).

(5) A. Geiger, J. Magnes, R. M. Taylor, and M. Veralli, *Amer. J. Physiol.*, **177,** 138(1954).

(6) A. Geiger and J. Magnes, *ibid.*, **149,** 517(1947).

(7) C. Allweis and J. Magnes, *J. Neurochem.*, **2,** 326(1958).

(8) A. Geiger, *Physiol. Rev.*, **38,** 1(1958).

(9) R. J. White, M. S. Albin, and J. Verdura, *Science*, **141,** 1060 (1963).

(10) D. D. Gilboe, W. W. Cotanch, and M. B. Glover, *Nature*, **202,** 399(1964).

(11) *Ibid.*, **206,** 94(1965).

(12) G. Benzi, F. Berté, A. Crema, and G. M. Frigo, *J. Pharm. Sci.*, **56,** 1349(1967).

(13) G. Benzi, F. Berté, E. Arrigoni, and L. Manzo, *ibid.*, **58,** 885(1969).

(14) G. Benzi, L. Manzo, M. De Bernardi, A. Ferrara, L. Sanguinetti, E. Arrigoni, and F. Berté, *ibid.*, **60,** 1320(1971).

(15) A. Faulconer, Jr., *Anesthesiology*, **13,** 361(1952).

(16) D. D. Gilboe, M. B. Glover, and W. W. Cotanch, *Amer. J. Physiol.*, **213,** 11(1967).

(17) U. Gottstein, A. Bernsmeier, and I. Sedlmeyer, *Klin. Wochenschr.*, **41,** 943(1963).

(18) W. A. Himwich and H. E. Himwich, *J. Neurophysiol.*, **9,** 133(1946).

(19) J. S. Meyer, F. Gotoh, M. Akiyama, and S. Yoshitake, *Circulation*, **36,** 197(1967).

(20) U. Gottstein, K. Held, H. Sebenning, and G. Walpurger, *Klin. Wochenschr.*, **43,** 965(1965).

(21) J. S. Meyer, T. Sawada, A. Kitamura, and M. Toyoda, *Circulation*, **37,** 1036(1968).

(22) W. E. Huckabee, *Amer. J. Cardiol.*, **12,** 663(1963).

(23) D. Stetten and Y. J. Topper, *Amer. J. Med.*, **19,** 96(1965).

[1] Personal communications from J. Cahn *et al.*, Institute for Experimental Therapy and Clinical Research, Montrouge, France.

(24) C. Alexander, P. J. Cohen, H. Wollman, J. C. Smith, M. Reivich, and R. A. Van der Molen, *Anesthesiology*, **26**, 624(1965).
(25) A. Moretti and G. Arcari, *Atti Congr. Soc. Sci. Farm. Appl.*, 1971.
(26) S. Gudbjarnason and R. J. Bing, *Biochim. Biophys. Acta*, **60**, 158(1962).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 19, 1971, from the *Department of Pharmacology, University of Pavia, Pavia, Italy.*
Accepted for publication November 10, 1971.
▲ To whom inquiries should be directed.

Chymotrypsin Inhibition of Muscular Contraction

ANWAR A. HAKIM

Abstract □ The effects of chymotrypsin and its various pharmaceutical preparations on skeletal, smooth, and cardiac muscles were tested. Chymotrypsin inhibited the development of adenosine 5′-triphosphate-induced tension by glycerol-extracted fibers and caused the relaxation of contracted fibers. When adenosine 5′-triphosphate-contracted muscle fibers were exposed to chymotrypsin and then washed, the muscle fibers failed to respond to the addition of the nucleotide triphosphate. If added to Tyrode's solution containing strips of tensed guinea pig gut, crystalline chymotrypsin or its various pharmaceutical preparations caused loss of tone, tension, and peristaltic movements. The enzyme preparations also inhibited the response of the gut strip to histamine or serotonin. When a perfusing rat heart was subjected to chymotrypsin, the enzyme caused an immediate short-term increase in cardiac tone and a series of abnormal contractions. These changes continued until the heart failed. Ultrasonication of chymotrypsin increased its hydrolytic activity on low molecular weight substrates and abolished its effects on muscle contraction.

Keyphrases □ Chymotrypsin—inhibition of adenosine 5′-triphosphate-induced muscular contraction, effect of ultrasonification on physicochemical and pharmacological properties □ Muscular contraction, adenosine 5′-triphosphate induced—inhibition by chymotrypsin □ Ultrasonication—effect on physicochemical and pharmacological properties of chymotrypsin

Chymotrypsin, a proteolytic enzyme, is of therapeutic interest as an anti-inflammatory agent. It is extensively employed to reverse inflammation, resorb edema, and liquify or localize suppurative exudation (1–6). For intramuscular administration, it is used as a suspension in sesame oil or as an aqueous solution. For oral administration, a purified enzyme concentrate is incorporated into tablets. The action of chymotrypsin has been attributed to the catalytic conversion of profibrinolysin to fibrinolysin (4, 5), a characteristic reaction of both chymotrypsin and trypsin (6).

Studies from these laboratories led to the isolation of a toxic glycoprotein from *in vitro* scalded human skin, which was shown to inhibit specifically the formation of adenosine 5′-triphosphate-induced contraction by glycerol-extracted rabbit muscle fibers (7, 8). This inhibitory activity of the glycoprotein was destroyed by incubating the glycoprotein with collagenase. The enzyme itself did not influence the development of adenosine 5′-triphosphate-induced tension. To extend these studies, the effects of chymotrypsin and trypsin were investigated. In these experiments, crystalline chymotrypsin and its various pharmaceutical forms were tested *in vitro* on the physiological properties of glycerol-extracted rabbit psoas muscle fibers, guinea pig gut, and isolated rat heart. When added to the bathing medium, chymotrypsin or its various pharmaceutical forms inhibited the formation of adenosine 5′-triphosphate-induced tension by muscle fibers. If added to the medium with adenosine 5′-triphosphate-contracted fibers, chymotrypsin or its various pharmaceutical forms caused immediate loss of tension. They also inhibited the response by guinea pig gut strips to histamine or serotonin and resulted in loss of tone, tension, and peristaltic movements. If perfused into the rat heart, they caused an immediate short-term increase in cardiac tone, a series of abnormal contractions, and an enhanced heart failure.

Sonication of the enzyme increased its hydrolytic activity on low molecular weight, synthetic substrates and abolished its effect on muscular contraction.

EXPERIMENTAL

Materials—α-Chymotrypsin[1] was crystallized (9) three times to obtain a preparation with an activity of 1200 units/mg. on *N*-acetyl-l-tyrosine ethyl ester and of 25 units/mg. on *N*-benzoyl-l-arginine ethyl ester. A sterile solution[2] was obtained containing 5000 units of purified chymotrypsin/ml. of 0.9% sodium chloride. A purified enzyme concentrate[3] containing a chymotrypsin mixture with a potency on hemoglobin of 3065, on *N*-acetyl-l-tyrosine ethyl ester of 495, and on *N*-benzoyl-l-arginine ethyl ester of 2625 units/mg. was employed as supplied. Trypsin[4], with a potency of 4067 units/mg. on *N*-benzoyl-l-arginine ethyl ester, was used.

The glycerol-extracted rabbit psoas muscle fibers were prepared according to the method of Szent-Gyorgyi (10). The guinea pig gut strips were obtained from freshly sacrificed animals, cleaned, and washed thoroughly with Tyrode's solution. The toxic glycoprotein was prepared as reported earlier (7, 8).

Methods[5]—An assay system was developed to measure the action of drugs on muscular activities. For the skeletal muscle, the system employed glycerol-extracted rabbit psoas muscle fibers. The fibers were suspended from a 0.3-force transducer in 62 m*M* tromethamine–phosphate buffer of pH 7.5 containing 2.5 m*M* $MgCl_2$. In this chamber the fibers were allowed to incubate at 26° for 60 sec.; then adenosine 5′-triphosphate was added in 0.10-ml. aliquots of 3×10^{-2} *M* solution to induce tension. The tension was maintained for 2 min.; then chymotrypsin was added in the amounts stated for each experiment.

[1] Armour lot K450038.
[2] Chymar injectable, Armour Pharmaceutical Co.
[3] Armour lots 582 and 583.
[4] Armour lot K152045.
[5] In all these experiments, the tension developed was monitored with a model Grass polygraph with 7 P 1 preamplifier and recorded.