The method described here allows specific determination of small amounts of organic nitrates in plasma. Plasma levels of nitroglycerin, after sublingual administration of the substance in man using the method described here, have been published elsewhere (15). The results obtained with sublingual isosorbide dinitrate are given in Table II.

Use of the method described here for the study of orally given nitrates will hopefully lead to a better understanding of the problem of the long-term prophylaxis of angina pectoris by these products.

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Estimation of Changes Induced by Drugs in Cerebral Energy-Coupling Processes *In Situ* in the Dog

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Abstract □ Drug action on the cerebral energy-yielding sequences was evaluated *in vivo via* the changes in the energy charge potential of the cerebral adenylate system and also the brain lactate and pyruvate systems. The two series of parameters were estimated before, during, and after hypoxemia in the hypovolemic, hypotensive beagle dog. The drugs were bemegride (6.4×10^{-4} *M*), nicergoline (1×10^{-4} *M*), and dipyridamole (2.5×10^{-4} *M*), which were perfused into the carotid artery at 0.5 ml./min. for 3 or 6 min. Bemegride reduces the energy charge, while dipyridamole and nicergoline increase the depressed cerebral energy charge potential. Nicergoline requires glucose and oxygen and is sensitive to malonate and cocaine.

Keyphrases □ Drugs—effects on cerebral energy-yielding processes, changes in charge potential of adenylate, lactate, and pyruvate systems using bemegride, nicergoline, and dipyridamole, beagle dogs □ Cerebral metabolism—effect of bemegride, nicergoline, and dipyridamole on energy-yielding processes, effect on adenylate, lactate, and pyruvate systems, beagle dogs □ Energy charge, cerebral—changes induced by bemegride, nicergoline, and dipyridamole before, during, and after hypoxemia in hypovolemic, hypotensive beagle dogs □ Metabolism, cerebral—drug-induced changes in energy-coupling processes, bemegride, nicergoline, and dipyridamole effects, beagle dogs □ Bemegride—effects on cerebral energy-coupling processes, beagle dogs □ Nicergoline—effects on cerebral energy-coupling processes, beagle dogs □ Dipyridamole—effects on cerebral energy-coupling processes, beagle dogs

Quantification *in vivo* of the action of drugs that can interfere in the cerebral energy-yielding sequences or that can modify cerebral reactions at metabolic branch points is a problem involving theoretical and methodological considerations. Since several experimental conditions such as anesthesia, hypothermia, or hypercapnia can produce large changes in the cerebral oxygen metabolic rate without disrupting the energy balance (1–3), changes in oxygen consumption cannot be used to define the action of a drug on the cerebral energy state. On the other hand, not all of the factors regulating the cerebral blood flow have been clarified, so flow changes cannot readily be used to evaluate drug action on energy states.

Furthermore, recent results have demonstrated that the phosphocreatine and lactate concentrations and the

lactate–pyruvate ratio are influenced by the intracellular pH, even if hypoxia is absent (4), so the lactate–pyruvate ratio must be used with caution. Therefore, the changes in the energy charge of the adenylate system were estimated.

The adenosine monophosphate level may be important in determining whether glycolysis or gluconeogenesis will predominate (5, 6), and the balance between the adenine nucleotide concentrations may be a major regulating factor at every point where a metabolite is partitioned between energy-yielding and energy-demanding or energy-storing processes (7–9). This is stated in terms of the energy charge of the adenylate pool (10, 11), a parameter that is intended to quantify the energy state of the cell. The control of adenosine triphosphate-regenerating sequences and, especially, regulation of interaction between adenosine triphosphate regeneration and processes that utilize adenosine triphosphate must clearly respond to the energy balance of the cell rather than to the concentration of a single metabolite (12). Then any drug-induced increase or decrease in the cerebral energy charge potential will be the result of the power to increase or decrease the rates of reactions that control adenosine triphosphate regeneration and to decrease or increase the rates of reactions controlling adenosine triphosphate use; both of these changes counteract the modifications in the energy charge of the brain.

This report concerns the drug-induced changes in the cerebral energy charge of the dog with normal tension, in hypovolemic hypotension, during control conditions, during hypoxia, and during recovery after hypoxia. Bemegride, nicergoline, and dipyridamole were chosen because of their metabolic action (13–18).

METHODS

Animals and Anesthesia—The experiments were carried out on 48 male beagle dogs at 240–360 days of age and weighing from 10.4 to 13.2 kg. Before the experiments the dogs were maintained under the same environmental conditions ($22 \pm 1°$, relative humidity = $60 \pm 5\%$) and were fed only on standard diet as powder (before 60 days of age) or as pellets (after 60 days of age), with water *ad libitum*.

The surgical procedure was performed on animals preanesthetized with urethan (0.4 g./kg. i.p.). Electrical activity of the brain (as portrayed by electroencephalography) was used to determine the degree of anesthesia (19), which was induced and maintained only during the surgical procedure by chloralose (30–40 mg./kg. i.v.). Because anesthetics affect labile phosphates and the extra- and intracellular brain lactate and pyruvate concentrations (2, 3, 20–23), electroencephalographic (EEG) pattern restoration indicated the removal of the anesthetic from the brain before the start of the experiment on cerebral energy metabolism. The dogs were artificially ventilated with room air or a nitrogen–oxygen mixture with an intratracheal Warne tube. During the operative procedure and the experiment, the animals were paralyzed by intravenous injection of gallamine triethiodide (2–3 mg./kg.) to permit unresisted artificial respiration and maintain a constant $PaCO_2$.

Operative Procedure—This procedure consisted mainly of isolation of both common carotid arteries, with ligature of all their branches except the internal carotid arteries and the superior thyroid ones. The zygomatic, maxillary, auricular, and supraorbital vessels were all occluded by ligature or compression. Each of the isolated superior thyroid arteries was cannulated with a polystan tube and connected by a two-way cannula to a perfusion apparatus (to infuse the intracarotid drug or saline solution) and to a glass syringe (to draw arterial blood samples).

The head was fixed to a head holder with the confluence of the cerebral venous sinuses approximately 10 cm. higher than the heart. Monopolar electrodes were inserted in the left and right frontal, parietal, and occipital areas. Heated thermocouples were placed on the brain surfaces to evaluate the changes in cortical blood flow. The flow probe was placed obliquely in the parietal cortex, with the tip adjacent to the cortical gray mantle. The small hole in the skull for the probe was carefully sealed to prevent untoward ambient heat exchange (24). After a longitudinal incision, a 2–2.5-cm. diameter hole was made in the frontoparietal area contralateral to the thermistor flow probe. A plastic funnel was fitted into the hole and the skin was sutured tightly around the funnel (25, 26); subsequently, the plastic funnel was sealed with a rubber stopper and thermally insulated.

Both femoral arteries were cannulated: one connected to a physiological pressure transducer and the other to a heparinized Wolfe bottle to withdraw arterial blood from the systemic circulation. The Wolfe bottle was connected to a femoral vein to infuse the drained blood into the systemic circulation. Connection was made by a large silicone-coated pararubber cannula joined to a peristaltic pump.

Cerebral blood flow, EEG, systemic pressure, and arterial P_{O_2} and P_{CO_2} were measured (using anaerobic samples from the superior thyroid artery). The rectal temperature was monitored with a telethermistor, and intermittent heating was applied to keep the dog's temperature as close to 37° as possible.

These parameters were recorded using a 12-channel recorder.

Design of Experiment—Previous research on the diphosphopyridine nucleotide concentration in the intact brain, as well as on the adenosine triphosphate or phosphocreatine concentration in the tissues, has shown changes for inspired oxygen concentrations below 7% (27–29). On the other hand, some studies have indicated the absence of changes in labile tissue phosphates with 4–5% oxygen in the inspired gas mixture (30). When the arterial P_{O_2} was reduced below 35 mm. Hg, the phosphocreatine content in the brain was reduced, but there were not even small changes in the adenylate system until the P_{O_2} was reduced below 25 mm. Hg. In any case, PaO_2 could be reduced to below 20 mm. Hg without causing major changes in the adenosine phosphates as long as the mean arterial blood pressure was held close to normal levels (4). Also, the cerebral circulation (31, 32) and the brain concentration of labile phosphate (33–36) are not significantly changed until the arterial hypotension results in a cerebral perfusion pressure of about 40 mm. Hg. Nevertheless, P_{O_2} below 25 mm. Hg causes changes in the adenosine phosphates to be exaggerated by even small decreases in the blood pressure (4). Therefore, the hypoxia experiments were carried out on beagle dogs in hypovolemic hypotension.

After the operative procedure, the EEG was followed until the anesthetic had been removed, and then a period of 30 min. was allowed before the hypotension was induced. During this period the cerebral vascular reactivity was tested by administration of an 8% carbon dioxide–air mixture through the respirator; the experiment was continued only if cerebral blood flow was increased, which indicated a qualitatively appropriate response to carbon dioxide in the cerebral vessels (24). Subsequently, the dogs were slowly bled from the arterial femoral cannula into a heparinized Wolfe bottle. A mean blood pressure of 60 mm. Hg was attained within 8–20 min., and then cautious arterial withdrawals or venous infusions by peristaltic pump were made so that the mean blood pressure was held as constant as possible for 25 min. Normally, the blood pressure could be held at the specified level to within ± 4 mm. Hg; if unduly large variations occurred or if a substantial part of the blood had to be reinfused to keep the blood pressure constant, the dog was discarded (36). The induced arterial hypotension changes $PaCO_2$ and the hemoglobin concentration. The controls had a mean blood pressure of 112 ± 8 mm. Hg, and the working mean blood pressure was 60 ± 4 mm. Hg; in this range the carbon dioxide tension decreased from 41 ± 4 to 36.5 ± 3 mm. Hg, while the hemoglobin concentration decreased from 18.4 ± 0.45 to 14.6 ± 0.94 g./100 ml.

The experiments were carried out as follows.

1. Evaluation of the drug action in curarized dogs with the systemic blood pressure within the normal range of 100–140 mm. Hg and with controlled ventilation with room air. The drug was perfused for 6 min. into the carotid artery at the rate of 0.5 ml./min., at the molar concentration indicated below.

2. Evaluation of the drug action in curarized dogs during and after arterial hypoxemia. The three periods in each experiment were

Table I—Curarized Beagle Dog: Cerebral Cortex Levels of Lactate, Pyruvate, Adenosine Triphosphate (ATP), Adenosine Diphosphate (ADP), and Adenosine Monophosphate (AMP), Expressed in Micromoles per Gram of Wet Tissue, after 6 min. of Intracarotid Perfusion (0.5 ml./min.) with Saline Solution, Nicergoline, Dipyridamole, and Bemegride

—Intracarotid Substance	Perfusion— Molar Concentration	Number of Dogs	Lactate, μmoles/g.	Pyruvate, μmoles/g.	Lactate / Pyruvate	ATP, μmoles/g.	ADP, μmoles/g.	AMP, μmoles/g.	ATP / ADP	ATP / AMP	Energy Charge Potential[a]
Saline solution	—	3	1.46 ±0.08	0.092 ±0.001	16.37 ±0.83	2.19 ±0.04	0.46 ±0.04	0.07 ±0.01	4.80 ±0.41	32.06 ±6.61	0.89 ±0.03
Nicergoline	1×10^{-4}	3	1.63 ±0.05	0.091 ±0.001	18.03 ±1.64	2.27 ±0.16	0.41 ±0.06	0.11 ±0.02	5.82 ±1.21	20.40 ±2.19	0.88 ±0.02
Dipyridamole	2.5×10^{-4}	3	1.51 ±0.19	0.094 ±0.011	17.07 ±4.24	2.22 ±0.08	0.41 ±0.03	0.11 ±0.04	5.42 ±0.40	21.20 ±1.19	0.89 ±0.04
Bemegride	6.4×10^{-4}	3	4.95[b] ±0.55	0.125[b] ±0.002	39.65[b] ±1.21	1.22[b] ±0.04	0.51 ±0.07	0.87[b] ±0.08	2.53[b] ±0.43	1.43[b] ±0.16	0.57[b] ±0.03

[a] Calculated as [(ATP) + 0.5 (ADP)]/[(ATP) + (ADP) + (AMP)]. [b] Significant difference ($p < 0.05$) from the value after saline solution perfusion, calculated by t test.

usually: (*a*) a steady-state period of hypovolemic hypotension of 10 min. during room air ventilation; (*b*) 12 min. of acute hypoxia induced with controlled $PaCO_2$ by altering the inspired oxygen concentration with a 6% oxygen–nitrogen mixture in the respirator (in some cases, saline perfusion or drugs were given during the last 6 min. of hypoxia); and (*c*) 3 min. of recovery of respiration with room air, and perfusion through one of the thyroid superior arteries with saline solution or drugs.

3. Evaluation of drug action after hypoxia and hypoglycemia in curarized dogs in hypovolemic hypotension. Fasting dogs were prepared in the same manner as already described. During the steady-state period, 800–1200 units of regular insulin was injected intravenously; 40–60 min. later, the dog was given 6% oxygen–nitrogen gas mixture.

The following drugs were perfused through the superior thyroid arteries at 0.5 ml./min. for 3 or 6 min.: (*a*) 4-methyl-4-ethyl-2,6-dioxopiperidine (bemegride), 6.4×10^{-4} *M;* (*b*) 1,6-dimethyl-8β-(5-bromonicotinoylhydroxymethyl)-10α-methoxyergoline tartrate (nicergoline or nimergoline), 1×10^{-4} *M;* (*c*) 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (dipyridamole), 2.5×10^{-4} *M;* (*d*) sodium malonate, 5×10^{-2} *M;* and (*e*) cocaine hydrochloride, 5×10^{-3} *M*. These concentrations were chosen in accordance with the clinical doses employed in man: 20–80 mg. of bemegride; from 2–4 to 10–15 mg. of nicergoline, and from 12.5–25 to 50–100 mg. of dipyridamole.

Analytical Techniques—At the set time, the motor area of the cortex was frozen *in situ* by pouring liquid nitrogen into the plastic funnel fitted into the cranial vault; the ventilation with room air or oxygen–nitrogen mixture was continued for at least 2 min. after the beginning of the regional brain freezing. A portion of the frozen brain was chiseled out using a rotating cold hollow tube (15-mm. i.d.) during continuous irrigation with liquid nitrogen. The frozen cerebral material was then immersed in liquid nitrogen for 10–15 min. It was then extracted with perchloric acid. The initial extraction was performed at from −10 to −15°, using frozen 3 *M* perchloric acid (4, 21), while the subsequent steps were carried out at 0–5° until a neutral perchlorate-free extract was obtained (4, 37, 38). The steps (4) were: extraction with 3 *M* perchloric acid at subzero temperatures, homogenization for 15 sec., additional extraction with 3% perchloric acid, centrifugation, neutralization of the combined extracts with 5 *N* KOH to pH 5.5, and separation of the potassium perchlorate by centrifugation. The neutralized, potassium perchlorate-free extract was then used for immediate analyses. Lactate, pyruvate, and adenosine mono-, di-, and triphosphates were assayed (37–39), a blank sample being run for each individual adenosine monophosphate analysis because the enzyme used contained adenosine monophosphate itself as a contaminant (21).

Estimation of Energy State of the Brain—The energy charge potential (10) was estimated assuming that the balance among concentrations of adenine nucleotides responds to the energy state of the cell rather than to the concentration of a single nucleotide (12).

The adenosine triphosphate regeneration or the adenosine triphosphate utilization in energy metabolism can be described by Scheme I:

$$2ADP + 2P_i \leftrightharpoons 2ATP + 2H_2O$$

Scheme I

while the reaction catalyzed by adenylate kinase is indicated as shown in Scheme II:

$$ATP + AMP \rightleftharpoons 2ADP$$

Scheme II

where ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate, and P_i = inorganic phosphate. The adenylate energy storage system may be described (Scheme III) by the sum of the processes indicated in Schemes I and II:

$$AMP + 2P_i \rightleftharpoons ATP + 2H_2O$$

Scheme III

The number of anhydride-bound phosphates per adenosine moiety varies from 2 to 0; division by 2 gives a parameter varying between 1 and 0, which has been named *energy charge* of the adenylate system.

The energy charge potential is defined by the ratio: half of anhydride-bound phosphates/concentration of the components of the adenylate system. The number of anhydride-bound phosphates is: 2(ATP) + 1(ADP) + 0(AMP); division by 2 gives: (ATP) + 0.5(ADP). Therefore, the energy charge is defined in terms of actual concentrations as:

$$\text{energy charge potential} = \frac{[(ATP) + 0.5(ADP)]}{[(ATP) + (ADP) + (AMP)]} \quad \text{(Eq. 1)}$$

2 —(division by 2)→ 1 → full charge: only ATP present

⇧

energy charge potential

⇩

0 —(division by 2)→ 0 → complete discharge: only AMP present

When only adenosine triphosphate and adenosine monophosphate are evaluated, Eq. 1 can be expressed as:

$$\text{energy charge potential} = \frac{(ATP)}{[(ATP) + (AMP)]} \quad \text{(Eq. 2)}$$

Equations 1 and 2 are compatible with Scheme III, but they are not identical because the concentrations of all three substances (adenosine mono-, di-, and triphosphates) contribute to the energy dynamic, but only two of these (adenosine di- and triphosphates) are involved in hydrolysis. Equation 1 was used in the present study.

Table II—Curarized Beagle Dog in Hypovolemic Hypotension: Cerebral Cortex Levels of Lactate, Pyruvate, Adenosine Triphosphate (ATP), Adenosine Diphosphate (ADP), and Adenosine Monophosphate (AMP), Expressed as Micromoles per Gram of Wet Tissue; Arterial PaO_2 and $PaCO_2$; and Relative Cortical Blood Flow in Various Experimental Conditions (the Rate of the Intracarotid Perfusion was 0.5 ml./min.)

Experimental Conditions	Intracarotid Perfusion: Substance	Intracarotid Perfusion: Molar Concentration	Number of Dogs	PaO_2, mm. Hg	$PaCO_2$, mm. Hg	Relative Cortical Blood Flow[a]	Lactate, µmoles/g.	Pyruvate, µmoles/g.	Lactate/Pyruvate	ATP, µmoles/g.	ADP, µmoles/g.	AMP, µmoles/g.	ATP/ADP	ATP/AMP	Energy Charge Potential[b]
Control condition[c]	Saline solution	—	5	96.4 ±2.3	36.5 ±1.3	100	2.28 ±0.10	0.122 ±0.009	18.85 ±0.67	2.28 ±0.06	0.45 ±0.03	0.09 ±0.01	5.20 ±0.42	27.64 ±4.54	0.89 ±0.01
Hypoxia[d] 2 min.	Saline solution	—	3	45.0 ±4.4	37.8 ±1.2	144[e] ±10	2.87 ±0.15	0.140 ±0.014	20.64 ±0.98	2.46 ±0.09	0.41 ±0.05	0.09 ±0.02	6.18 ±0.69	30.22 ±7.87	0.90 ±0.05
6 min.	Saline solution	—	3	27.3[e] ±1.3	36.0 ±1.8	180[e] ±13	7.60[e] ±0.56	0.209[e] ±0.017	36.20[e] ±1.90	2.22 ±0.44	0.44 ±0.04	0.12 ±0.01	5.10 ±0.47	18.35 ±1.72	0.87 ±0.01
12 min.	Saline solution	—	3	19.3[e] ±0.7	40.2 ±0.9	222[e] ±16	23.03[e] ±1.72	0.267[e] ±0.025	88.60[e] ±13.29	1.68[e] ±0.16	0.52 ±0.05	0.64[e] ±0.17	3.23[e] ±0.30	3.23[e] ±1.15	0.68[e] ±0.06
After 3-min. period of recovery of room air ventilation	Saline solution	—	3	98.3 ±2.8	37.8 ±1.0	143 ±13	11.87 ±1.23	0.166 ±0.025	78.50 ±22.99	1.98 ±0.09	0.53 ±0.05	0.37 ±0.05	3.84 ±0.49	5.68 ±1.09	0.78 ±0.02
	Nicergoline	1×10^{-4}	3	96.0 ±3.2	35.8 ±1.0	162 ±20	12.80 ±1.36	0.176 ±0.025	75.00 ±13.92	2.16 ±0.10	0.45 ±0.06	0.13[f] ±0.02	4.88 ±0.44	16.91[f] ±1.15	0.87[f] ±0.02
	Dipyridamole	2.5×10^{-4}	3	97.7 ±2.2	36.3 ±1.3	151 ±14	8.93 ±0.81	0.128 ±0.017	73.73 ±14.30	2.12 ±0.08	0.46 ±0.05	0.17[f] ±0.02	4.69 ±0.31	12.72[f] ±1.83	0.85[f] ±0.01
	Bemegride	6.4×10^{-4}	3	98.0 ±2.3	36.2 ±1.3	181[f] ±16	30.53[b] ±0.82	0.298[f] ±0.025	103.17[f] ±7.31	1.36[f] ±0.07	0.54 ±0.04	0.97[f] ±0.10	2.54[f] ±0.18	1.45[f] ±0.23	0.57[f] ±0.03

[a] Expressed as percent of the control value equal 100. [b] Calculated as [(ATP) + 0.5 (ADP)]/[(ATP) + (ADP) + (AMP)]. [c] Room air controlled ventilation and 6 min. of saline solution perfusion. [d] Induced by controlled ventilation with 6% oxygen–nitrogen mixture. [e] Statistical difference ($p < 0.05$) *versus* the value of the control condition. [f] Statistical difference ($p < 0.05$) *versus* the value after 3-min. period of recovery of room air ventilation and intracarotid perfusion with saline solution.

Obviously the generalization proposed by the quantification of the drug action on the energy-yielding sequences by the cerebral energy charge of the adenylate pool is necessarily oversimplified because little satisfactory information is available as to: (*a*) the extent of the importance of barrier compartmentation as compared with kinetic compartmentation, the selective permeabilities and transport properties being modulated by both the energy charge and the levels of specific metabolites on the sides of the barrier; (*b*) the interactions between feedback modifiers and energy charge in regulation of biosynthetic sequences and in production of storage compounds, or in regulation of sequences that regenerate adenosine triphosphate and supply primary biosynthetic intermediates; and (*c*) the sequences involved in the control of the phosphopyridine nucleotide system.

Statistical Analysis—The *t* test was applied to the differences in all instances.

RESULTS AND DISCUSSION

Drug Action during Room Air Ventilation—Table I shows that a curarized normal beagle dog receiving room air responds to intracarotid perfusion (0.5 ml./min. for 6 min.) with nicergoline (1×10^{-4} *M*) and dipyridamole (2.5×10^{-4} *M*), with no change in the levels of lactate, pyruvate, and labile phosphates in the cerebral cortex or in the cerebral energy charge; perfusion with bemegride (6.4×10^{-4} *M*) increases the lactate, pyruvate, and lactate–pyruvate ratio in the cerebral cortex. Previous observations on curarized monkeys (14) have shown that arterial and venous cerebral blood lactate rapidly increase by about twofold after bemegride injection. In the present study, the animals were curarized so any effect of bemegride on muscular contraction and muscle metabolism could be ignored. Furthermore, intracarotid perfusion with bemegride (Table I) induced a decrease in the adenosine triphosphate level and an increase in the adenosine monophosphate concentration in the cerebral cortex, the adenosine diphosphate level being quite unchanged. The energy charge potential was significantly reduced. These data are in agreement with the observation (39, 40) that agents inducing seizures are able to lower the levels of adenosine triphosphate and phosphocreatine in the brain, even if the seizure activity may be associated with unchanged levels of cerebral high energy compounds (47).

Drug Action during Hypoxia and Hypotension—*Influence of Arterial Hypoxemia*—Table II shows that hypoxia induced by a 6% oxygen–nitrogen mixture caused PaO_2 in hypotensive beagle dogs to fall rapidly, while the $PaCO_2$ varied little because of the controlled ventilation. The cerebral cortical blood flow increased significantly at PaO_2 below about 50 mm. Hg. Furthermore, there were changes in the lactate, pyruvate, and lactate–pyruvate ratio in the brain at PaO_2 below about 30–40 mm. Hg, but there were no significant variations in the cerebral energy charge potential until PaO_2 fell below 20 mm. Hg (Table II). This finding indicates that the brain lactate and pyruvate can change at PaO_2 levels which are compatible with unchanged energy charge, which confirms that the cerebral lactate content or production can increase at oxygen tensions compatible with unchanged levels of labile phosphates (27, 30, 36, 41–43).

Different anatomical regions of the brain vary in sensitivity to energy deprivation (44) and the time course of energy deprivation (45, 46). In fact, gross irreversible damage from total asphyxia is limited almost exclusively to the brain stem (45), while in partial asphyxia damage to the cortex is the major finding (46). In the present research, during oxygen–nitrogen mixture respiration, the cerebral energy charge potential on the cortex of the frontal, parietal, and occipital areas was simultaneously evaluated in three dogs. At $PaO_2 = 19.3 \pm 0.7$ mm. Hg, the mean values were 0.66 ± 0.07, 0.69 ± 0.05, and 0.70 ± 0.04, respectively, which are not significantly different.

After 12 min. of hypoxia, PaO_2 rose rapidly to the control value following normal ventilation and perfusion for 3 min. through the superior thyroid artery with saline solution (Table II). The increased cortical blood flow fell when the gas was replaced by room air but only by about 45% within the 3 min. of observation. Also, the lactate, pyruvate, and adenosine triphosphate levels and the energy charge potential only partially recovered within the 3 min. (Table II).

Effect of Pharmacological Treatment with Drugs—After hypoxia, room air ventilation and perfusion through the superior thyroid

Table III—Curarized Beagle Dog in Hypovolemic Hypotension: Cerebral Cortex Level of Adenosine Triphosphate (ATP), Adenosine Diphosphate (ADP), and Adenosine Monophosphate (AMP), Expressed as Micromoles per Gram of Wet Tissue, in Various Experimental Conditions (Rate of Intracarotid Perfusion was 0.5 ml./min.)

Experimental Conditions	Intracarotid Perfusion: Substance	Intracarotid Perfusion: Molar Concentration	Number of Dogs	ATP, μmoles/g.	ADP, μmoles/g.	AMP, μmoles/g.	ATP/ADP	ATP/AMP	Energy Charge Potential[a]
After 12-min. period of hypoxia[b]	Saline solution	—	3	1.68 ±0.16	0.52 ±0.05	0.64 ±0.17	3.23 ±0.30	3.23 ±1.15	0.68 ±0.06
	Nicergoline	1×10^{-4}	3	1.63 ±0.12	0.57 ±0.06	0.59 ±0.12	2.90 ±0.15	3.07 ±0.72	0.68 ±0.04
After 3-min. period of recovery of room air ventilation	Saline solution	—	3	1.98[c] ±0.09	0.53 ±0.05	0.37[c] ±0.05	3.84 ±0.49	5.86[c] ±1.09	0.78[c] ±0.02
	Nicergoline	1×10^{-4}	3	2.16[c] ±0.10	0.45 ±0.06	0.13[c,d] ±0.02	4.88 ±0.44	16.91[c,d] ±1.15	0.87[c,d] ±0.02
	Nicergoline + malonate	1×10^{-4} 5×10^{-2}	3	1.71[d] ±0.15	0.49 ±0.01	0.64[d] ±0.17	3.49 ±0.39	3.14[d] ±0.92	0.68[d] ±0.06
	Nicergoline + cocaine	1×10^{-4} 5×10^{-3}	3	1.60[d] ±0.10	0.54 ±0.04	0.74[d] ±0.11	3.02 ±0.38	2.30[d] ±0.47	0.65[d] ±0.04
After 3-min. period of recovery of room air ventilation in hypoglycemia[e]	Nicergoline	1×10^{-4}	3	1.63[d] ±0.17	0.54 ±0.03	0.67[d] ±0.19	3.02 ±0.25	2.43[d] ±1.06	0.67[d] ±0.06

[a] Calculated as [(ATP) + 0.5 (ADP)]/[(ATP) + (ADP) + (AMP)]. [b] Induced by controlled ventilation with 6% oxygen–nitrogen mixture. [c] Statistical difference ($p < 0.05$) *versus* the value after 12-min. period of hypoxia. [d] Statistical difference ($p < 0.05$) *versus* the value after 3-min. period of recovery of room air ventilation and intracarotid perfusion with saline solution. [e] Induced by intravenous injection of 800–1200 units of regular insulin.

artery with bemegride (6.4×10^{-4} *M* at 0.5 ml./min.) for 3 min. (Table II) inhibited the cerebral metabolic restoration and decreased the adenosine triphosphate level and the energy charge potential, while the lactate and pyruvate concentrations and the adenosine monophosphate level increased later. Bemegride also inhibited the normal reduction in the cerebral blood flow occurring during the recovery with room air respiration. In fact, the residual enhancement in cortical blood flow after 3 min. of saline solution perfusion was about 45%; after bemegride perfusion, it was about 80%. During bemegride perfusion, no significant differences in PaO_2 or $PaCO_2$ were present relative to saline perfusion.

After hypoxia, recovery with room air ventilation, and intracarotid perfusion (0.5 ml./min.) with nicergoline (1×10^{-4} *M*) or dipyridamole (2.5×10^{-4} *M*) for 3 min. (Table II), the energy charge potential was significantly different from that induced by saline perfusion. The drugs decreased significantly the adenosine monophosphate concentration. Conversely, no action on the lactate and pyruvate levels or on the lactate–pyruvate ratio of the cortex was observed. During drug perfusion, no PaO_2 and $PaCO_2$ values significantly different from the saline case were found.

The mechanism was elucidated by perfusing nicergoline (1×10^{-4} *M*) at 0.5 ml./min. during hypoxia; the drug induced no significant change in the cerebral energy charge (Table III). Furthermore, during the recovery with room air ventilation after hypoxia, nicergoline (1×10^{-4} *M*) was tested during simultaneous perfusion with sodium malonate (5×10^{-2} *M*) or cocaine (5×10^{-3} *M*) at 0.5 ml./min. for 3 min. Both agents blocked the effect of nicergoline action on the cerebral energy charge (Table III). On the other hand, hypoglycemia inhibited the action of nicergoline on the energy charge potential of the brain during recovery with room air ventilation after hypoxia (Table III).

Mode and Mechanism of Drug Action—It was possible to distinguish drugs that reduce the energy charge (*e.g.*, bemegride) from ones that increase it (*e.g.*, nicergoline and dipyridamole).

Bemegride affects the energy metabolism of the brain in the following four respects:

1. After injection of bemegride in animals immobilized with curare, appearance of spike activity in the EEG is correlated with a marked increase in the cerebral blood flow and cerebral metabolic rate for oxygen (13).

2. Cerebral glucose consumption and cerebral blood lactate levels increase significantly, showing a condition compatible with increases in aerobic and anaerobic glycolyses during seizure activity induced by bemegride (14).

3. Electric shock and drugs inducing seizures are known to decrease the levels of adenosine triphosphate and phosphocreatine in brain (39, 40).

4. The eventual reduction in cerebral levels of high energy phosphates during seizures reflects the discrepancy between the high energy consumption and the normal resynthesis of labile phosphates, as occurs during hypoxia localized in the brain itself (39, 40, 48).

In the present study, intracarotid perfusion with bemegride in beagle dog induced: (*a*) an increase in the lactate and pyruvate levels of the cortex and a decrease in the energy charge, the adenosine triphosphate being decreased and the adenosine monophosphate being increased in the brain tissues; and (*b*) inhibition of the normal restoration of the cerebral energy charge potential after hypoxia. All these results support the hypothesis that bemegride may exert a hypoxic effect on the brain because of a discrepancy between the energy-demanding processes and the oxygen that the increased cerebral blood flow can supply. In any case, bemegride reduced the stored energy in spite of the increase in some pharmacodynamic (EEG and cerebral blood flow) or metabolic parameters (cerebral glucose consumption and cerebral metabolic rate for oxygen).

There are three notable points about the action of drugs that raise the depressed energy charge potential of the brain: (*a*) nicergoline (as well as dipyridamole) is inactive during the control condition, the cerebral energy charge being normal; (*b*) nicergoline is inactive during hypoxemia; and (*c*) malonate, cocaine, and hypoglycemia inhibit the action of nicergoline on cerebral labile phosphates during the recovery of room air ventilation after hypoxia. The mechanism of action of ergoline derivatives is only partially elucidated by these observations. As regards malonate inhibition, it should be noted that: (*a*) the well-known inhibitory effect on succinate oxidation makes malonate a potent inhibitor of the citric acid respiratory cycle (49), (*b*) potassium-stimulated brain respiration is highly malonate sensitive (50, 51), and (*c*) the stimulation of metabolism by a changed K^+/Ca^{+2} balance is inherently associated with effects at the brain cell membrane (52). Malonate inhibition of nicergoline action was observed in the present study, and it supports the hypothesis that the drug increases the K^+/Ca^{+2} balance and the associated activity in the citric acid cycle, because ionic movements at cell membranes occur by the initiation of reactions involving energy change in the adenosine phosphate system. The hypothesis concerning nicergoline interference in the K^+/Ca^{+2} balance is supported also by the nicergoline antagonism against the Ca^{+2}

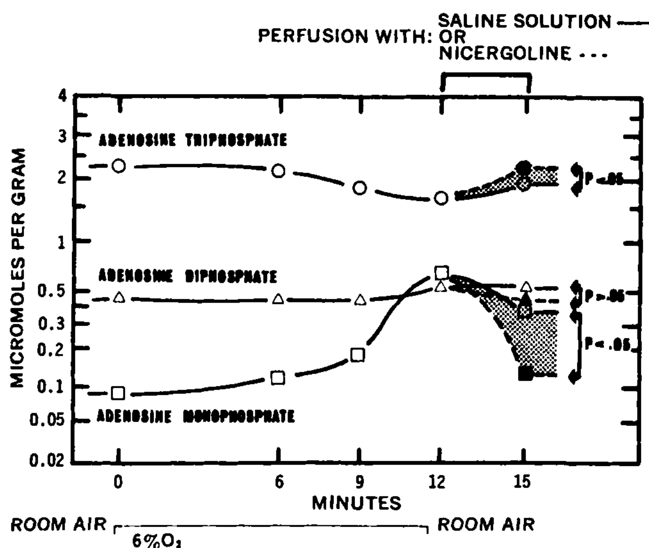

Figure 1—*Variation in the cerebral cortex levels of adenosine mono-, di-, and triphosphates induced in beagle dogs by hypoxia and recovery with room air ventilation and intracarotid perfusion (0.5 ml./min. for 3 min.) with saline solution or nicergoline* (5×10^{-4} M). *The abscissa shows the time of observation. The numbers by the lines for the concentrations represent the probability levels of the difference between the saline solution perfusion and the nicergoline perfusion. Each point represents the average value for three dogs.*

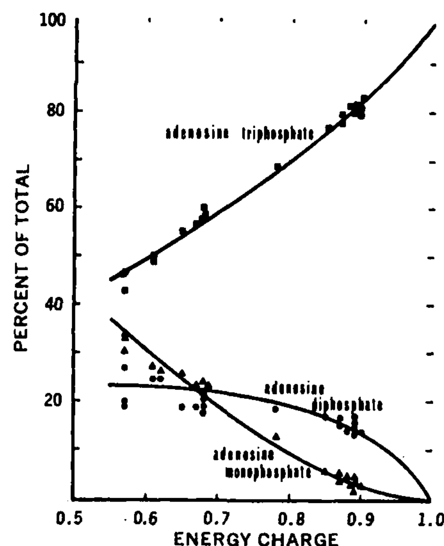

Figure 2—*Cortical motor region of the brain of beagle dog in various experimental conditions: relative concentrations of the adenine nucleotides as a function of energy charge.*

accumulation by epinephrine (53) and by cocaine inhibition of the nicergoline effect on brain energy-coupling processes. In fact, cocaine acts in a manner similar to Ca^{+2}, its presence resulting in an effective diminution of a K^{+}/Ca^{+2} ratio at the brain cell surface (52).

The estimation of adenine nucleotides in the cortical motor region of the brain shows that hypoxia in the hypovolemic, hypotensive dog leaves the adenosine diphosphate level little changed, while the decrease in adenosine triphosphate is related to an increase in adenosine monophosphate (Fig. 1), which confirms the previous suggestion that the level of adenosine monophosphate may be considered a measure of the stress upon the brain (39). After 3 min. of recovery with room air ventilation, the adenosine monophosphate concentration varied inversely with the adenosine triphosphate concentration, the adenosine diphosphate being little changed. Intracarotid nicergoline perfusion (1×10^{-4} *M*, 0.5 ml./min. for 3 min.) enhanced significantly the decrease in adenosine monophosphate and the increase in adenosine triphosphate, while adenosine diphosphate remained unchanged (Fig. 1). This confirms that the ordered changes among the adenine nucleotides are a consequence of the constant sum of adenosine mono-, di-, and triphosphates and the slight changes in adenosine diphosphate, which together force an inverse relationship between adenosine monophosphate and adenosine triphosphate. The relative proportions of the individual adenine nucleotides are functions of the cerebral energy charge (Fig. 2).

CONCLUSIONS

It is difficult to quantify the action of drugs upon cerebral energy-yielding sequences because there is doubt as to the parameter to measure and the conditions inducing change in the energy-coupling processes.

The oxygen consumption and the cerebral blood flow cannot be used, because the cerebral metabolic rate for oxygen and the cerebral blood flow can be modified without change or with opposite change in the cerebral energy balance. On the other hand, the factors regulating the cerebral blood flow have not yet been clarified (1–3, 47, 54). In the present *in vivo* study, the lactate and pyruvate levels of the brain and the cerebral energy charge *via* the adenosine mono-, di-, and triphosphates were evaluated. There was some difference between these two groups of parameters since the lactate and pyruvate of the brain can change at PaO_2 values which are compatible with unchanged levels of the stored energy. Therefore, it seems advisable to interpret the lactate–pyruvate changes with caution, since no other parameter of the cerebral energy state can be used.

The changes in the energy state of the brain induced by drugs are best evaluated from the cerebral energy charge potential in the adenine nucleotide pool, but two points should be stressed:

1. A change in the energy charge of the motor area does not imply that an equal change has occurred in the whole cerebral tissue or in any other important local region of the brain.

2. A change in energy charge evaluated from the adenine nucleotide pool does not imply that an equal change has occurred in other substrates and, particularly, in the creatine phosphate system. Nevertheless, several enzymes that are involved in energy-yielding sequences, or that catalyze reactions at metabolic branch points, are affected by adenine nucleotides. The pattern of the responses suggests that they participate in the regulatory interactions that must underlie orderly metabolic integration. The creatine phosphate can be affected by hypoxemia before changes in the adenosine phosphate pool have occurred; in fact, at PaO_2 of about 35 mm. Hg, the cerebral phosphocreatine level is significantly reduced, but there are no changes in the adenosine mono-, di-, or triphosphate concentration (4). In any case, it must also be stressed that isolated adenosine triphosphate values give relatively restricted information (54).

The cerebral energy state is remarkably resistant to various experimental conditions. Thus, PaO_2 could be reduced to below 20 mm. Hg without causing major changes in adenosine phosphates as long as the mean arterial blood pressure is held close to normal levels, while more pronounced changes are induced in hypotensive animals (4). In the present research, the action of the drugs on the cerebral energy-coupling processes *in situ* was evaluated by inducing a hypoxic condition in the hypovolemic, hypotensive dog.

Bemegride lowered the cerebral energy charge, while nicergoline and dipyridamole increased the depressed energy charge. The drugs showed significant effects on the relative proportions of the individual components of the adenylate system, which are related to the energy-yielding, energy-demanding, and energy-storing processes, the cerebral blood flow being unchanged or changing in the opposite direction. Nicergoline resembles dipyridamole in being inactive in the normal brain. Its action on the cerebral energy, as depressed by hypoxemia and hypotension, is antagonized by malonate, cocaine, and hypoglycemia.

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