The Effect of Pyridoxine and Pyridoxal on the Circulatory Response of Rats to Microwave Irradiation

Hyperthermia is readily induced in rats by microwave irradiation. We have previously demonstrated that cardiac output, heart rate, and arterial blood pressure increase after exposure to such irradiation. Peak levels are recorded when rectal temperature reaches 40.5°C. The pattern of response may be modified by interference with the sympathoadrenal system[1,2], suggesting participation of endogenous vasoactive amines. Conjugation of certain amines with pyridoxal eliminates the ability of the amines to stimulate the heart and increase blood pressure[3]. It seemed possible, therefore, that pyridoxine or pyridoxal could modify cardiovascular responses to microwave irradiation if these responses were mediated by endogenous vasoactive amines.

Methods. Thirty albino rats, anesthetized by intraperitoneal injection of sodium pentobarbital (25 mg/kg), were placed in the supine position. A femoral artery was cannulated for the measurement of arterial blood pressure with a Statham P23d pressure transducer. Arterial blood temperature was measured by a thermistor housed in a PE 10 polyethylene catheter and placed in the aorta by way of a carotid artery. A jugular vein was cannulated for administration of drugs and for injection of cold indicator solution, the dilution of which served as a basis for measurement of cardiac output by a thermodilution method[4]. Heart rate was measured from lead II of an electrocardiogram. The electrocardiogram, blood pressure and the indicator dilution curves were recorded simultaneously on a direct writing oscillograph. Rectal temperature was monitored with a thermistor. Measurements were taken in triplicate.

Rats in groups of ten were exposed to microwaves (2,450 Mc. continuous wave, 0.08 W/cm^2) at a distance of 5 cm. The output head of the generator was centered over the sternum and upper abdomen. When the rectal temperature reached 40.5°C, the irradiation was discontinued and measurements of cardiac output, heart rate and blood pressure were repeated. One group in which no drug was given provided normal values. In a second group, after control measurements were made, 10 rats were given pyridoxine (10 mg/kg) intravenously in a saline vehicle. Measurements were repeated, followed by irradiation of the animals in the manner described above. Measurements were again recorded upon termination of exposure at rectal temperature 40.5°C. The third group of 10 rats were treated exactly as the second group except that pyridoxal (10 mg/kg) was administered instead of pyridoxine. In analysis of the data, stroke volumes and total peripheral resistances were calculated. The statistical significance of the differences in responses was determined with the T-test.

Results. The data are summarized in the Table. In the group of untreated rats the microwave irradiation was followed by significant increases in cardiac output, heart rate, blood pressure and calculated stroke volume. Total peripheral resistance decreased. In contrast, the cardiac output of rats which were given pyridoxine or pyridoxal before microwave irradiation did not increase significantly despite a significant increase in heart rate. Mean arterial blood pressure increased. The increase in calculated total peripheral resistance was not significant ($P > 0.05$). The pyridoxine and pyridoxal in the dose used did not cause significant alterations in hemodynamics at normal body temperatures, i.e. before exposure to the microwaves. Exposure time did not vary between groups and averaged 10 min.

Discussion. These data demonstrate that compounds of the vitamin B_6 group modify the circulatory response of the anesthetized rat to microwave induced hyperthermia. The mechanism by which pyridoxine or pyridoxal prevents the rise in cardiac output is not evident in these experiments. The dose employed is well within maximal doses tolerated by rats (LD_{50} = 3 g/kg)[5]. Furthermore, pyridoxine is present in cardiac tissue of rats in appreciable amounts[6]. It is noteworthy that a significant increase in heart rate follows irradiation in the rats treated with pyridoxine or pyridoxal. Since cardiac acceleration during the hyperthermic state also persists

1 T. COOPER, T. PINAKATT, M. JELLINEK, and A. W. RICHARDSON, Am. J. Physiol. *202*, 1171 (1962).

2 T. COOPER, T. PINAKATT, M. JELLINEK, and A. W. RICHARDSON, Aerospace Med. *33*, 794 (1962).

3 H. F. SCHOTT and W. G. CLARK, J. biol. Chem. *196*, 449 (1952).

4 A. W. RICHARDSON, T. COOPER, and T. PINAKATT, Science *135*, 317 (1962).

5 W. S. SPECTOR, *Handbook of Biological Data* (W. B. Saunders Co., Philadelphia 1956), p. 229.

6 H. K. MITCHELL and E. R. ISBELL, *Studies on the Vitamin Content of Tissues*, II. Austin, Tex. U. Tex., Publication 37-40 (1942).

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Group	No. of animals	Weight in g	Status	Cardiac output (ml/min)	Heart rate per min	Stroke volume (ml)	Mean arterial blood pressure (mm Hg)	Total peripheral resistance (units)
Untreated	10	429 ± 13	C	118.5 ± 6.2	372 ± 9	0.32 ± 0.02	124 ± 3	1.03 ± 0.59
			E	176.6 ± 8.3[a]	415 ± 8[a]	0.43 ± 0.02[a]	139 ± 4[a]	0.71 ± 0.11[c]
Pyridoxine (10 mg/kg)	10	426 ± 27	C	132.5 ± 3.6	357 ± 8	0.37 ± 0.01	125 ± 2	0.96 ± 0.04
			P	129.7 ± 4.4	340 ± 14	0.39 ± 0.02	123 ± 3	0.96 ± 0.05
			E	134.0 ± 6.4	381 ± 8[d]	0.35 ± 0.02	137 ± 3[a]	1.04 ± 0.06
Pyridoxal (10 mg/kg)	10	433 ± 10	C	130.0 ± 4.0	341 ± 11	0.38 ± 0.02	125 ± 2	0.96 ± 0.03
			P	128.6 ± 4.5	310 ± 14	0.41 ± 0.02	123 ± 5	0.96 ± 0.04
			E	135.3 ± 4.8	381 ± 11[d]	0.37 ± 0.02	142 ± 5[b]	1.08 ± 0.06

Values expressed as mean ± S.E. C = control. P = after administration of agent (before irradiation). E = after microwave irradiation. P values reflect significance level from control observations. [a] < 0.001, [b] < 0.01, [c] < 0.02, [d] < 0.05.

after vagotomy and adrenalectomy[2] and reserpinization[1], it would seem possible that persistent acceleration is a direct effect of heating.

The added vitamin B_6 compounds may interact with or block the action of endogenous vasoactive amines liberated during the hyperthermia stress of the microwave irradiation. Support for this possibility is found in reports that adrenalectomy eliminates the increase in cardiac output due to microwave irradiation whereas ganglionic blockade[2] or reserpinization[1] merely reduces the increase in output; and that the catecholamines and histamine content of the plasma increase[7] during induced hyperthermia of a similar degree.

It is usually conceded that the administration of vitamins in excess of the amount provided in the usual daily minimal requirement is of little consequence. The absence of any demonstrable effect from the administration of the vitamin at normal body temperature reinforces this view. However, the inhibition of the increase in cardiac output during a rapidly induced change in status emphasized other possible functions of these biologically active compounds. These studies do not indicate whether the effect obtained during hyperthermia is beneficial. The duration of the effect has not yet been delineated[8].

Résumé. L'administration intraveineuse de pyridoxine et pyridoxal (10 mg/kg) à des rats anesthésiés inhibe l'augmentation du rendement du coeur. En même temps, une hyperthermie est provoquée par l'irradiation du corps entier avec des ondes de longueur minime (2,450 Mc. c.w. 0.08/w/cm²). Cet effet peut être attribué à l'interaction de ces composés d'amines vasoactifs libérés pendant l'irradiation.

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[7] P. N. SYMBAS, M. JELLINEK, T. COOPER, and C. R. HANLON, Arch. int. Pharmacodyn. Therap. *150*, 132 (1964).

[8] Aided by Grants HE-06312, HE-06702 and HE-K3-5616, USPHS and John A. Hartford Foundation and by Contract NR 102-362, Office of Naval Research.

Changes Effected in vitro upon some Morphological Properties of Particles of Virus BAI-Strain A

The work of BEARD et al. has supplied us with basic facts about the structure and properties of virus BAI-strain A particles[1–6]. Our previous papers dealing with the pleiomorphism of these particles[7–8] have pointed out the relation between the functional state of leukemic cells, the stage of the leukemic process, and the occurrence of spherical or tailed forms of virus particles isolated from blood plasma. This paper deals with experiments directed towards influencing the form of these virus particles in vitro.

Materials and methods. White Leghorn chickens 17–20 days old, chosen for their susceptibility to virus BAI-strain A (which causes myeloblastosis when applied intravenously[6,9,10]), were used in our experiments.

The primary material used for the preparation of the individual plasmas was the cytoquantitatively defined blood taken from experimental animals[11]. The heparinized blood was sedimented at 1500 *g* for 30 min and at 2000 *g* for 30 min and the supernatant was used further.

Experiments directed towards influencing the form of virus particles were carried out on two or more aliquot amounts of the same plasma. This method was chosen in order to preserve the virus in its natural medium, and to eliminate in this manner any influence that a purification in other media could have effected. To the first amount of plasma (used for reference) was added the solution of tris-(hydroxymethyl)-aminoethane-KCl-$MgSO_4$ in an amount necessary to obtain a final concentration of 1 m*M* of all decisive components. The same solution further containing the substrate (e.g. adenosine-triphosphate-Na) or the inhibitor was added to the experimental portion of the plasma itself, to obtain the final concentration of 1 m*M* as well. Incubation time in all experiments was 7 min at 37°C.

The virus was sediménted from the plasma at 34000 *g*. The sediment of virus particles thus obtained was immediately resuspended in a 0.15*M* solution of ammonium carbonate and ammonium acetate of pH 7.0. The specimens for the electron microscope were prepared by spraying this solution onto a collodion membrane with a high pressure spray-gun[12]. The fixation in OsO_4 vapours was followed by Au-Pd alloy shadow-casting.

The following compounds were used in our experiments:

ATP – adenosine-5'-triphosphoric acid – Sigma Co.
ADP – adenosine-5'-diphosphoric acid – Boehringer
AMP – adenosine-5'-monophosphoric acid – Fluka AG.
Tris – tris-(hydroxymethyl)-aminoethane
PCMB – *p*-chloro-mercuri benzoate Na
PMB – phenyl-mercuri borate
NEM – N-ethylmaleimid
MJA – iodo-acetic acid
Atebrine – chinacrinum hydrochloricum
Chloropromazine (Largactil) – Specia, Paris.

[1] D. BEARD, E. A. ECKERT, T. Z. CZÁKY, D. G. SHARP, and J. W. BEARD, Proc. Soc. exp. Biol. Med. *75*, 533 (1950).
[2] D. G. SHARP, E. A. ECKERT, D. BEARD, and J. W. BEARD, J. Bacteriol. *63*, 151 (1952).
[3] J. W. BEARD, Poultry Sci. *35*, 203 (1956).
[4] J. W. BEARD, Texas Reports on Biology and Medicine *15*, 627 (1957).
[5] R. A. BONAR and J. W. BEARD, J. Nat. Cancer Inst. *23*, 183 (1959).
[6] J. W. BEARD, *Progress in Hematology* (1962), vol. III, p. 105.
[7] P. BARTL, J. ŘÍMAN, and F. ŠORM, Exper. *19*, 635 (1963).
[8] P. BARTL, J. ŘÍMAN, and J. KORB, Proc. III. Europ. Reg. Conf. on Electron Microscopy, Prague (1964), vol. B, p. 373.
[9] J. W. BEARD, Ann. N.Y. Acad. Sci. *68*, 473 (1957).
[10] E. A. ECKERT, D. BEARD, and J. W. BEARD, J. Nat. Cancer Inst. *12*, 447 (1951).
[11] J. ŘÍMAN, Folia biol., Praha *10*, 331 (1964).
[12] R. C. BACKUS, J. appl. Phys. *23*, 159 (1952).