Volume 288, number 1,2, 244–246 FEBS 10084 August 1991

ADONIS 001457939100797D

Significant correlations between atmospheric spectra according to Baumer and the in vitro incorporation of [^{3}H]thymidine into the nuclear DNA of C6-glioma cells

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Received 24 June 1991

Significant correlations between certain spectra of atmospherics (spherics) according to Baumer (a.t.B), i.e. naturally occurring electro-magnetic impulses in the range of 4–50 kHz, and several diseases or biological parameters have been published earlier. Now we show that there exists a highly significant negative correlation ($r = -0.61$, $P > 0.004$) between the occurrence of 28 kHz impulses (a.t.B.) and the in vitro incorporation of thymidine into the nuclear DNA of C6-glioma cells. The positive correlation with the 10 kHz impulses (a.t.B.) ($r = 0.39$), however, is statistically not significant ($P > 0.055$).

C6-glioma cell; Atmospheric spectrum (a.t.B.); Correlation

1. INTRODUCTION

A biological effect of weather-related events has long been suspected and correlations between the onset of diseases, accidents, or suicides and the atmospheric conditions have been found. Attempts to identify individual meteorologic factors responsible for the pathophysiological effects, however, have often failed when 'classical' climatic parameters like temperature, humidity or barometric pressure were correlated with biological phenomena. A biotrophic effect of atmospherics ('spherics'), i.e. naturally occurring electromagnetic pulses in the range of 4–50 kHz, has been suspected for a while but could not be proven due to the lack of recording equipment. In 1980 H. Baumer and J. Eichmeier, however, published the construction of a very sophisticated measuring device. As the notation of the impulse frequencies used for the correlations originates from the Baumer apparatus we call them correlation 'according to Baumer' (see also discussion). Using this machinery constructed by Baumer and Eichmeier [1] it was found that atmospherics measured according to Baumer (see later) were not equally distributed within the above mentioned range: 6, 8, 10, 12 and 28 kHz impulses (a.t.B). Further, we found that not only certain dynamic weather processes were correlated with different impulse spectra [2], but also the onset of certain diseases like epileptic fits [3], heart infarcts [4], and sudden deafness [5].

The origin of spherics has long been controversial. An earlier theory contributed them only to world-wide lightning activity [6]. The modern interpretation which corresponds to the definition of atmospherics by the World Meteorological Organization (1955) favors besides lightnings also counter movements of air masses 400–500 km round the observation point as the causes of spheric generation [7,8].

Animal experiments have been performed to reveal mechanisms by which spherics (a.t.B.) may exert their biological effects. It could be shown that the inflammation reaction of rats after carrageenan injection correlated with the rates of 8 and 10 kHz (a.t.B.) atmospherics [9]. Recently a significant correlation was found between atmospherics and the in vivo incorporation of [^{3}H]thymidine into the nuclear DNA of liver cells of normal mice [10]. The current investigation was designed to evaluate a correlation of atmospherics (a.t.B.) on thymidine incorporation in vitro into the DNA of cells from the tumor line C6-glioma.

2. MATERIALS AND METHODS

2.1. *Electrical measurement technique*

The device to measure the spectrum of atmospherics (a.t.B.) has previously been described in detail [3], US Patent No. 4631957; 30.12.1986; US Patent No. 4684951, 4.8.1987). Briefly, atmospherics were picked up by two 7-element ferrite antennae with pre-amplifiers

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 Published by Elsevier Science Publishers B.V.

installed 17 metres above ground level. Co-axial cables were used to transmit the signals to the measuring devices. Incoming impulses were sorted into appropriate channels on the basis of their frequencies. Digital filters served to closely define the bandwidths of interest at 10 kHz ± 700 Hz and at 28 kHz (+1.5 kHz, −3 kHz) appropriate to the known distribution of frequencies in the spectrum of atmospherics (a.t.B.). Impulses or groups of impulses so selected were counted in one hour intervals. The frequency sequence of the impulses was delivered to the printer in three steps. The first step corresponds to an activity until 0.3 Hz and the second to an average activity. The third step is defined as that step by which the frequency sequence of the impulse is 2.5 Hz or greater. The mean value was derived as the average of the hourly mean of the three levels. This value was used as measure of the atmospheric activity.

Using this equipment, impulses generated within an area of an approximate radius of 400 to 500 km were detectable. Therefore, we were concerned principally with ground wave propagation: ionospheric reflections play only a subordinate role.

2.2. *Cell culture conditions*

C6-glioma cells were grown at 37°C in Dulbecco's minimal essential medium (DMEM), supplemented with 10% fetal calf serum (FCS, Boehringer Mannheim, Germany) in a humidified atmosphere of 5% CO_2 and 95% room air in plastic Petri dishes in an incubator Heraeus Typ EK-CO_2. The cells were subcultivated 2–3 times per week. For each experiment, two confluent cultures obtained 2 days after subcultivation were used. Cells were harvested by a brief exposure to 0.05% trypsin, 0.02% EDTA in phosphate-buffered saline (PBS), resuspended in FCS-containing media, pooled and equal amounts of cells seeded into four Petri dishes. This well-standardized procedure led to a cell yield of about 10^7 cells/dish the following day, when two of the cultures were used to study [^{3}H]thymidine incorporation.

2.3. *Thymidine incorporation*

[^{3}H]Thymidine incorporation was evaluated exactly between 10-11 a.m. at 24 arbitrarily selected days between September 6, 1988 and June 6, 1989. To do so, the medium was removed, and cells washed two times with PBS. Then 5 ml fresh DMEM containing 10% FCS and [^{3}H]thymidine (3.7×10^4 Bq/ml medium) were added. After 1 h incubation at 37°C the medium was carefully removed, cultures washed 2 times with PBS, and all cells collected using a rubber policeman in as little PBS as possible. The samples were kept frozen until analysis of DNA content and [^{3}H]thymidine incorporation. The value of the specific radioactivity as measure for the [^{3}H]thymidine incorporation into the cells was calculated from the quotient of the radioactivity and the amount of DNA, expressed in dpm/μg DNA. For that purpose the DNA was extracted according to the method of Weinbren and Woodward [11]. Briefly the cells were thawed and suspended in 3 ml refrigerated 0.25 M perchloric acid (PCA). After destroying the RNA of the obtained sediment with 0.5 ml 0.5 M NaOH the precipitate was boiled twice with even 3 ml 0.5 M PCA. From the combined supernatants the radioactivity and the DNA content were determined. Radioactivity was measured in a liquid scintillation counter (Betamatic B. Kontron Instruments GmbH; Eching, Germany). DNA was determined according to the dye reaction of Burton [12].

Table I

Correlations of atmospherics (a.t.B.) observed between 06:00 and 12:00 a.m. at 24 arbitrarily selected days between September 6, 1988 and June 6, 1989 with [^{3}H]thymidine incorporation rates into C6-glioma cells

Frequency (kHz)	Spearman rank order correlation		Linear regression	
	r	P	r	P
10	0.39	<0.006	0.37	<0.074
28	−0.61	<0.004	−0.62	<0.001
10-28	0.61	<0.004	0.62	<0.001

3. RESULTS

The experiments had been performed in Munich, Germany between September 6, 1988 and June 6, 1989. Although the experimental protocol had been strictly standardized, the incorporation rates for labelled thymidine varied considerably over the experimental days, whereas the variation was minimal between cultures from the same day. The minimal amount of [^{3}H]thymidine incorporated into nuclear DNA was 11.4×10^3 dpm/μg DNA on April 19, 1989 as compared to a maximum of 62.7×10^3 dpm/μg DNA on September 21, 1988. Accordingly, values of the 10 kHz atmospherics (a.t.B.) registered during 6 hours (see section 2) varied between 4 on April 19, 1989 and 12 on September 21, 1989. The equivalent of 28 kHz atmospherics (a.t.B.) observed at three experimental days (September 7 and 14, 1988; May 6, 1989) was only one as compared to a maximum of 11 registered April 4, 1989.

Correlations of the daily measurements of thymidine incorporation and spheric activity revealed a highly significant negative correlation with the occurrence of 28 kHz atmospherics (a.t.B.) using the Spearman rank order correlation test ($r = -0.61$ $P > 0.004$, Table I). A linear regression analysis yielded comparable results ($r = -0.62$, $P < 0.001$, Fig. 1) suggesting a linear relationship between 28 kHz atmospherics (a.t.B.) and thymidine incorporation. Substracting the values of 28 kHz atmospherics (a.t.B.) from the values of 10 kHz atmospherics observed between 6 a.m. and 12 a.m. of identical days gave the same linear regression coefficient. 10 kHz atmospherics (a.t.B.) alone marginally missed the level of statistical significance in the Spearman rank order correlation test ($r = 0.39$, $P < 0.057$).

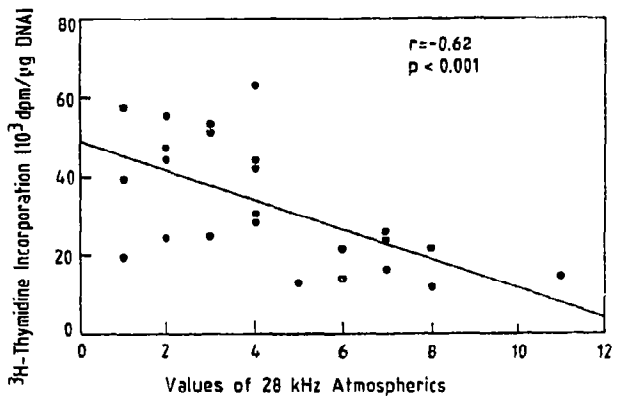

Fig. 1. Plot of values of 28 kHz atmospherics (a.t.B.) observed between 06:00 and 12:00 a.m. at 24 arbitrarily selected days between September 6, 1988 and June 6, 1989 against the [^{3}H]thymidine incorporation into the DNA of C6-glioma cells. Each point represents the mean value of the determinations of two samples of the same charge.

4. DISCUSSION

The results presented here demonstrate a significant, negative correlation between the occurrence of 28 kHz atmospherics (a.t.B.) and thymidine incorporation into nuclear DNA of C6-glioma cells. In the previous study on thymidine incorporation of normal mice liver cells, a positive correlation with 10 kHz atmospherics (a.t.B.) was observed, whereas 28 kHz spherics (a.t.B.) were not significantly correlated [10]. The correlation with the balance of s(10 kHz)-s(28 kHz) atmospherics (a.t.B.), however, was even more closely correlated than the number of 10 kHz spherics (a.t.B.) alone. The data, therefore, suggested an opposing effect of spherics (a.t.B) of the 10 and 28 kHz frequency range. This observation was confirmed by the present study. Again, at days with a frequent occurrence of 10 kHz atmospherics (a.t.B.) or with few 28 kHz atmospherics (a.t.B.) thymidine incorporation was elevated. Calculating the difference s(10 kHz)-s(28 kHz) in the present investigation however, did not improve the correlation.

10 kHz and 28 kHz atmospherics (a.t.B.) are closely related to meteorological changes as shown by the analysis of the daily atmospheric pattern during one year between May 1978 and April 1979 [2]. The data were also corrected for the daily pattern of the atmospheric pulse rates [13]. The authors found the 28 kHz atmospheric activity measured according to Baumer increased at days with vertical air movements and high-reaching turbulence, in particular. 10 kHz atmospherics were associated with an increased horizontal movement of air masses. From this information one may assume that thymidine incorporation is reduced mainly at days with little horizontal and massive vertical air movements, a situation typical for an approaching cold front.

Up to now, the device used to register atmospherics (a.t.B.) can only analyse the frequency ranges of the impulses. It is an open question whether a detailed study of the amplitudes of the spheric pulses might add significant information.

Up to now nobody knows what special signal of the impulses of atmospherics of the Baumer machinery is the actual origin of the biological effects. Therefore, we signify the atmospherics measured with the machinery 'according to Baumer' (a.t.B.) as long as this uncertainty continues.

By correlation one cannot prove causal connections. Perhaps the measured atmospherics (a.t.B.) are only suitable markers for another phenomenon causing the onset of certain diseases or biological parameters. To clarify this question, it is important to simulate all signals combined with the atmospherics (a.t.B.) just as they occur naturally. If the experiments with simulated atmospherics (a.t.B.) gave the same results as the present correlations it would be the working basis for investigating the mode of the atmospheric effects. To do so cell cultures are an appropriate trial.

A causal biometeorological effect of atmospherics (a.t.B.) would have considerable impact in medicine.

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