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Standardized Mental Stress in Healthy Volunteers Induced by Delayed Auditory Feedback (DAF)*

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Summary. Using delayed auditory feedback (delay 0.175 s) a standardized form of mental stress was investigated in 8 healthy male volunteers. After a resting period and a period of undelayed reading, the volunteers were exposed for 5 min to the DAF stress. During the DAF period heart rate increased by 10% and systolic and diastolic blood pressure increased by 9% and 18%, respectively. As a measure of acute sympathetic activation, plasma concentrations of norepinephrine and epinephrine rose by 68% and 49%, respectively. The activity of dopamine-β-hydroxylase in plasma was increased by 25%. From these results it can be concluded that the DAF procedure provides a suitable method for inducing a standardized mental stress in normal subjects, which can be measured as changes in biochemical and cardiovascular variables.

Key words: mental stress, sympathetic activation; delayed auditory feedback, plasma norepinephrine, plasma epinephrine, plasma dopamine-beta-hydroxylase

The results of psychopharmacological investigations in healthy volunteers often show inadequate reports of drug effects and only give an unsatisfactory indication of the therapeutic value of the substance examined [9].

The literature contains several methodological attempts to optimise the model value of such investigations in healthy volunteers. Subjects have often been selected according to their personality [17]. Furthermore, pharmacodynamic effects in healthy volunteers become measurable when they show a symptomatology which is above average but not pathological, e.g., border-line depressed subjects [7] or anxious subjects [1].

Another experimental approach consists in transferring healthy subjects into a state of reversible "psychic disturbance" by suitable measures, as far as ethically and medically acceptable, in order to study the effects of psychotropic drugs under such conditions.

Techniques such as induction of fear by using pictures [27] and unpleasant films [33], or induction of stress by noise [16], or by difficult arithmetical tasks [23], have contributed greatly in gaining information about the effect of psychotropic agents in healthy volunteers. Such "psychic disturbances", and the influence upon them of medication, have been assessed objectively by measurement of heart rate, blood pressure, skin resistance and other somatic variables. Associated biochemical changes in these types of investigations have seldom been taken into consideration [31].

Therefore, we accepted the following aims in the development of a standardized model for inducing mental stress in healthy volunteers:

1. Quantification of the applied stress
2. Objective assessment of the induced "psychic disturbance" by means of differential analysis of changes occurring in biochemical and cardiovascular variables.
3. Reproducibility of the results
4. Degree of tolerability and rapid reversibility of the "psychic disturbance".

As regards reproducibility, many of the models noted above are inadequate in that the effect of the

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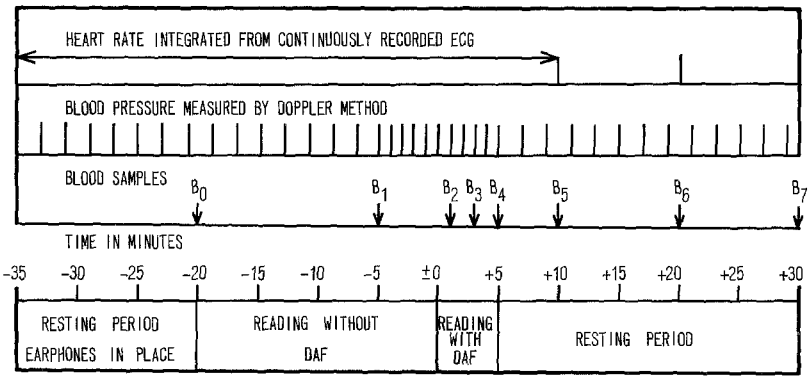

Fig. 1. Delayed Auditory Feedback (DAF) stress model Experimental protocol. In blood samples B_0–B_7 the following biochemical variables were measured: norepinephrine, epinephrine, dopamine-β-hydroxylase activity, lactate, glucose

stress stimulus weakens on repetition. On the other hand, the technique of *D*elayed *A*uditory *F*eedback [22, 15] causes a state of extreme psychic disturbance, which is only insignificantly reduced after repeated and continuous application [20].

Although the treatment of stuttering subjects with DAF is sometimes effective, it is experienced as extremely stressful by healthy volunteers. Speech is made difficult by the disturbed feedback which can only be overcome under great strain. The stress so induced differs from the other procedures mentioned because it is not produced by an external stimulus, which can lose its effect through habit, but that in the test subject the function of controlling and directing speach through feedback is disturbed.

The present study is an account of the application of DAF to induction of stress and of the initial results, i.e., rapidly occurring changes in sympathetic activity, as measured by an increased concentration of catecholamines and dopamine-β-hydroxylase (DBH) activity in plasma, and concomitant increases in heart rate and blood pressure.

Material and Methods

Delayed Auditory Feedback (DAF). In this technique, the normal auditory feedback of speech is drowned and disturbed by a timed, delayed feedback; the speaker hears everything he has spoken after a delay of 0.1 s or more. The apparatus used to produce DAF is simple. The test subject speaks a standard text into a microphone. The spoken text is recorded on an endless loop of magnetic tape, which is forwarded to a second play-back head and the recorded speech is then transferred back to the test subject via earphones.

In general a delay of 0.15 s is used to induce stress, whereas a longer delay is employed in speech therapy [4]. In the present studies a speech delayer (Zak, Simbach/Inn) with an adjustable delay of 0.175 s was used. Sections from Emmanuel Kant's book "Kritik der reinen Vernunft" were chosen as the text, because due to its complexity, it can hardly be memorised, and so it is suitable for repeated use.

Subjects. The study was carried out in 8 healthy male members of the volunteer panel of the Clinical Pharmacology Department, Hoechst AG, Frankfurt. Age 39.0 ± 4.7 years; height 178.3 ± 8.7 cm; weight 75.6 ± 4.7 kg; MPI neuroticism score according to Eysenck [10]: 28.4 ± 6.7. All values are expressed as mean ± standard deviation.

The subjects were informed about the nature, purpose and risk of the study and gave their written informed consent for their participation. They underwent medical and clinical chemical check-ups and were considered to be in good general health.

Trial Schedule (Fig. 1). The volunteers came to the trial in the morning after an overnight fast and remained fasting until the end of the study. To avoid physical and psychological influences, the subjects lay on beds in quiet, fully air conditioned rooms. Reading was facilitated by adjustment of the lying position.

An indwelling cannula for blood sampling [21] was placed in a cubital vein 30 min before B_0 (Fig. 1). The earphones were then placed in position.

The study was carried out according to a predetermined time schedule (Fig. 1).

The experimental period was divided into 4 sections:
- a *resting* period of 15 min with earphones applied,
- a *reading* period of 20 min *without* delay,
- a *reading* period of 5 min *with* delay (DAF stress) and
- a *resting* period of 25 min without reading and without earphones.

Blood samples (B_0–B_7) were taken at the time intervals shown in Figure 1.

Biochemical variables. The concentrations of the *catecholamines* epinephrine and norepinephrine were determined radioenzymatically, using the method of

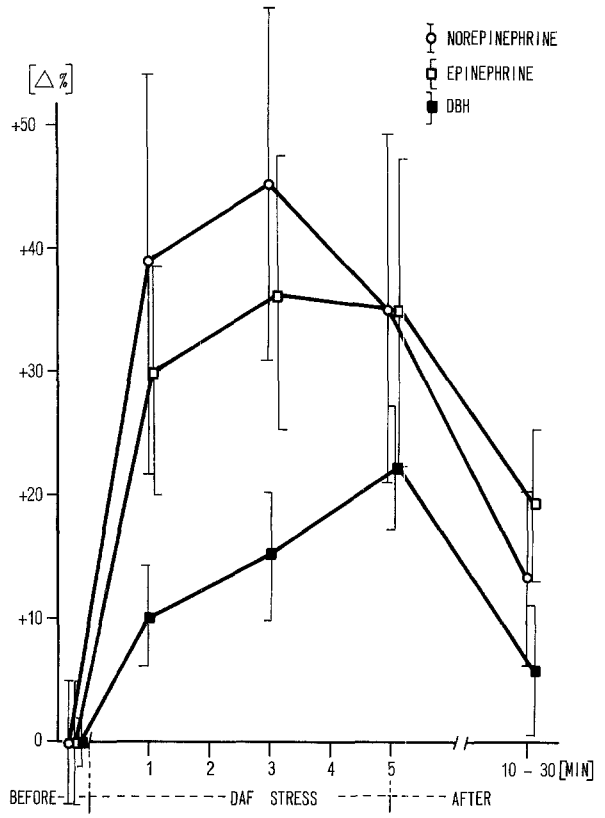

Fig. 2. Increase in catecholamine concentration and dopamine-β-hydroxylase activity (DBH) in plasma during DAF stress. Each point represents the mean ± SEM of 8 individual experiments expressed as △ % of the baseline values (reading period without feedback delay)
Baseline values: Norepinephrine 260 ± 14 [ng/l]
Epinephrine 84 ± 4 [ng/l]
DBH and 102 ± 2 [%]

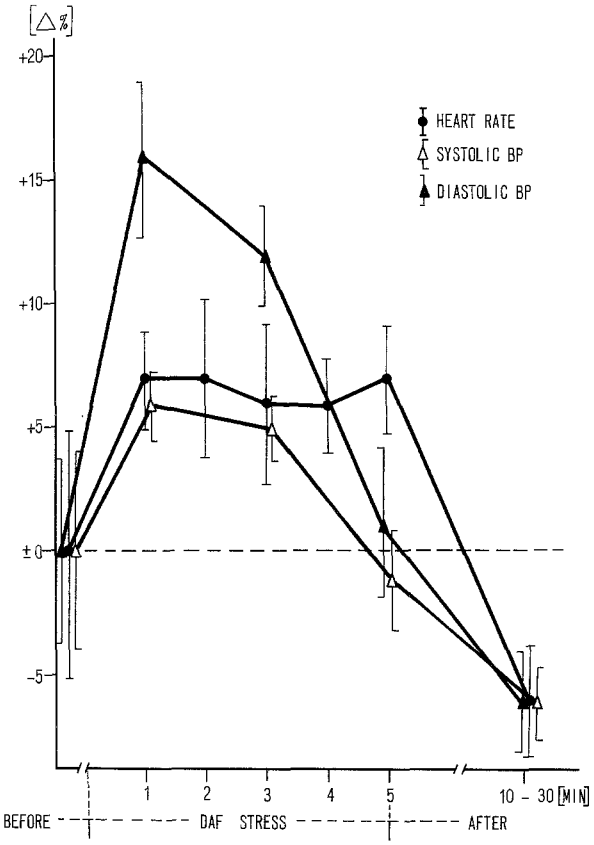

Fig. 3. Changes in heart rate and systolic and diastolic blood pressure during DAF stress. Each point represents the mean ± SEM of 8 individual experiments expressed as △ % of the baseline values (reading period without feedback delay)
Baseline Values: Heart rate 77 ± 4 [4min^{-1}]
Systolic BP 132 ± 5 [mm Hg]
Diastolic BP 95 ± 4 [mm Hg]

Passson and Peuler [26], i.e., after enzymatic methylation with catechol-O-methyltransferase and ^{3}H-S-adenosyl-methionine (spec. act. 8–9 Ci/mmol; NEN Dreieich). The activity of dopamine-β-hydroxylase (DBH; EC 1.14.2.1) in plasma was measured radiometrically using the method of Weinshilboum and Axelrod [32].

Glucose and lactate concentrations in blood were measured enzymatically by the methods of Stork and Schmidt [29] and Gutman and Wahlefeld [14], respectively.

Cardiovascular Variables. Blood pressure was measured ultrasonically (Arteriosonde 1217, Hoffmann La Roche AG, Bio-Electronics) and was recorded every 2 min on a linear potentiometric recorder. During and 5 min before the DAF-period blood pressure was measured every minute. Heart rate was measured continuously by means of the ECG from the beginning until 5 min after termination of the DAF-period. Two additional measurements were taken 15 and 25 min after the end of the DAF-period.

Results

DAF produces acute enhancement of sympathetic activity, which is rapidly reversible after termination of the stressful event.

The time course of the changes in sympathetic activity, as determined by DBH activity, and plasma norepinephrine and epinephrine during and after the DAF-period are shown in Figure 2. All three variables showed a pronounced rise even 1 min after the onset of DAF-stress. The maximum values also remained slightly increased during the following period of rest.

The time course of the changes in the cardiovascular variables (heart rate, systolic and diastolic

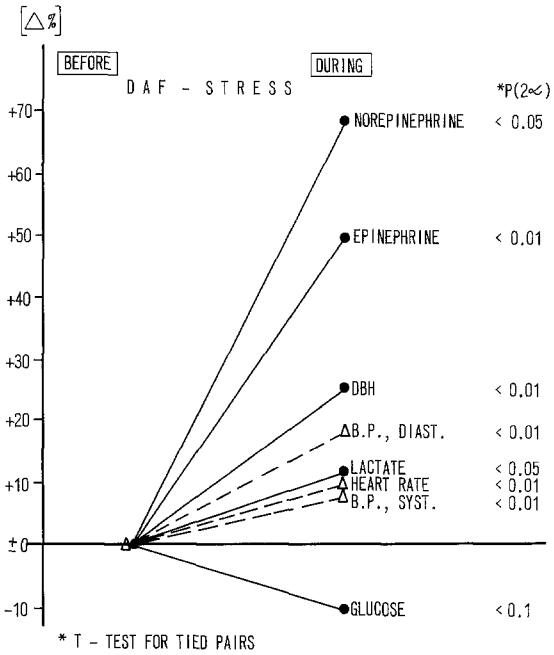

Fig. 4. Maximal change in biochemical and cardiovascular variables during DAF stress. Each point represents the mean of 8 individual experiments expressed as △ % of the baseline values (reading period without feedback delay). Levels of significance were calculated by a t-test for tied pairs.
●——— Biochemical variables
△– – – Cardiovascular variables

blood pressure) are shown in Figure 3. During the reading period without delay, all three variables increased by 6% as compared to the resting period (−35 up to −20 min; cf. Fig. 1).

The concentrations of epinephrine, norepinephrine and DBH activity in plasma did not differ significantly between the resting period and the reading period without DAF. Mean values of both periods (B_0 and B_1) were, therefore, used as baseline values (cf. Fig. 2).

From this level (baseline value), and according to the biochemical measures of sympathetic activation, within the 1st min of DAF stress an acute rise occurred in diastolic and systolic blood pressure, as well as in heart rate. The latter remained constant during the 5 min of DAF stress, whereas diastolic and systolic blood pressure decreased after the 1st minute of DAF stress. During the following resting period (cf. Fig. 1), the values found prior to the first reading period without delay were again obtained.

The maximal changes in all biochemical and cardiovascular variables measured are summarised in Figure 4. All changes induced by the DAF stress were significantly different from the corresponding baseline values (reading period without delay, cf. Fig. 1) with the exception of the changes in glucose concentration in blood.

Discussion

The method of delayed auditory feedback (DAF) appears to induce acute mental stress. It is an advantage of the technique that it can be standardized to a much higher degree than the other methods mentioned in the introduction. Furthermore, in contrast to most other methods as yet available for production of psychic stress, the stressful event lasts for only 5 min. During this experimental period the volunteers appeared to be strongly disturbed, i.e., steady reading suddenly changed into stammering when "reading without DAF" was unexpectedly switched to "reading with DAF".

The signs of mental stress could be quantified by measuring the cardio-vascular parameters of sympathoneuronal and sympatho-adrenal activation; heart rate and systolic and diastolic blood pressures had increased within 1 min after onset of DAF (Fig. 3). It appears, however, that even during the DAF period, sympathetic activation was counteracted, possibly by vagal impulses: the increased diastolic and systolic blood pressures tended to decrease during the last 2 minutes reaching values below those measured during the "pre-DAF" period 10–30 min after termination of DAF. As a quantitative measure of sympathetic activation, the overflow and release of catecholamines from the sympathetic nerve endings and from the adrenal medulla can be used. During prolonged mental stress an increase in urinary excretion of catecholamines, especially of epinephrine, has been demonstrated [8, 11, 12, 24].

The effects of short lasting stress, however, can be detected only by measuring changes in catecholamine concentration in plasma [19]. Changes in sympathetic activity can also be measured, at least under certain experimental conditions, by determination of dopamine-β-hydroxylase activity in plasma [2, 13, 18, 25, 28, 32].

In agreement with the measured changes in the cardiovascular variables throughout the DAF-period raised concentrations of epinephrine and norepinephrine were accompanied by significant increases in DBH activity in plasma (Fig. 2). In contrast to heart rate and blood pressure, raised catecholamine concentration could be measured 10–30 min after termination of DAF.

This may indicate that catecholamine release continues after the end of the period of stress. Similar results were obtained in animal experiments after physical stress [5].

The results during the 5 min DAF period are in agreement with those obtained in experiments lasting for hours: irrespective of its duration mental stress induces an activation of the sympatho-adrenal and of

the sympatho-neuronal system [8], whereas physical stress leads mainly to sympatho-neuronal activation, i.e., only the concentration of circulating norepinephrine and DBH is increased [28]. Only a small increase in plasma adrenaline was observed after physical exercise, e.g., by Christensen and Brandsborg [6].

The results depicted in Figure 4 show that, unlike to the measurements described above, metabolic changes induced by sympathetic activation, e.g., increased lactate and glucose concentration in blood, are much less sensitive indicators. The concentration of free fatty acids remained unchanged during the DAF period (results not shown).

The DAF model would only be a useful test of mental stress if it were reproducible in terms of the cardiovascular and biochemical parameters showing the same changes in the same volunteer after repeated application of the DAF test. We have recently confirmed this in a double-blind intra-subject comparison during 5 treatments with placebo, β-adrenoceptor blocking drugs and tranquilisers [30].

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