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Acute influence of cigarette smoke in platelets, catecholamines and neurophysins in the normal conditions of daily life

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Cigarette smoking is firmly linked to the occurrence of acute coronary events. In twenty-two healthy volunteers in normal conditions of daily life we studied the acute influence of smoking on the following parameters: beta-thromboglobulin, thromboxane B_2, epinephrine, norepinephrine, estrogen-stimulated neurophysin, and nicotine-stimulated-neurophysin. Our results show that in our population and following our protocol, smoking did not induce platelet activation, thromboxane formation, catecholamine release or estrogen-stimulated-neurophysin secretion. However, smoking did provoke a significant increase of nicotine-stimulated-neurophysin ($p<0{\cdot}05$) which reflects vasopressin increase and which might explain the high incidence of ischaemic accidents in cigarette smoking via the vasoactive properties of vasopressin.

Cigarette smoking is a well-recognised risk factor of coronary artery disease[1–3]. The mechanisms whereby smoking contributes to the development and ischaemic complications of coronary atherosclerosis are not well elucidated: smoking-induced catecholamines release[4] and platelet activation[5–6] have been proposed but without general agreement[7]. The present work tries to clarify these two points. In addition, we have studied the alteration of the two neurophysins.

The neuro-hypophysis of mammals contains vasopressin (AVP), oxytocin (OT) and their carrier proteins called neurophysins. There is considerable evidence that AVP and OT are released into the circulation, together with their specific neurophysin[8–10]. Recently, it was demonstrated that each hormone and its neurophysin originate from the same precursor[11]. AVP and OT have vasoactive properties and may play a role in triggering acute coronary events associated with cigarette smoking, since recent observations suggest that tobacco could act as a transient and reversible factor rather than via the promotion of atherosclerosis[12–14].

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The protocol used was specially designed to study these different variables when the subjects are under normal conditions of daily life, because smoking-associated coronary events occur usually in these conditions, and not in an experimental context.

Methods

POPULATION:

The studied population consisted of 22 healthy volunteers; 14 were males and 8 females with a mean age of 30 years (range: 18 to 56). The 22 subjects were regular smokers and were selected for their capacity to inhale tobacco smoke. Four out of the 8 females were taking oral contraceptives.

PROTOCOL

All the subjects smoked 2 cigarettes containing 1·2 mg of nicotine, at 10 a.m., without previous rest but after an overnight abstinence from smoking.

During the whole test, the subjects did not change their usual daily occupation, desk or laboratory work. Blood samples were drawn in all the subjects in the sitting position before smoking and about 20 min later by two different venipunctures. This time interval between blood samples was chosen in accordance with the study of

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Levine[4], in which this interval seemed to be the best time frame to detect the major change in platelet substances, and with the results of Cryer[5] which showed the highest catecholamine values between 10 and 20 min after smoking. The second venipuncture, performed on the opposite arm 10 min after the end of smoking, was preferred to butterfly sampling to avoid artefactual platelet activation[15] and to reproduce the possible puncture stress while mimicking normal daily life activity. In addition, in 5 subjects, blood samples were drawn at 5 min intervals during half an hour to study the catecholamine response to smoke as a function of time. In parallel, the pulse rate and systolic blood pressure were recorded and the double product was calculated in these 5 subjects. No haemodynamic measurements were done in the rest of the population.

PLATELET STUDY

Platelet function was evaluated by measurements of the two platelet-specific substances, beta-thromboglobulin (βTG) and thromboxane B_2 (TXB_2). βTG and TXB_2 plasma levels were measured by radioimmunoassay, with commercial RIA Kits (respectively, New England Nuclear, Boston, MA, USA, and Serono Diagnostics, Switzerland) by methods previously described[15].

RELEASE OF POSTERIOR HYPOPHYSIAL PEPTIDES

This was evaluated by determination of plasma levels of the two neurophysins. Each neurophysin is specifically associated with one neurohormone and has distinct electrophoretic mobilities, immunological properties, and responses to different stimuli. For instance, the vasopressin-associated neurophysin is released not only in response to variations in osmolarity but also to nicotine stimulation; therefore it is named nicotine-stimulated-neurophysin (NSSN). The oxytocin-associated-neurophysin is released in response to estrogen stimulation, and is named estrogen-stimulated-neurophysin (ESN)[16,17]. It is widely accepted that the plasma levels of neurophysins reflect the plasma levels of the associated neurohormones[8,16]. The two neurophysins were measured by radioimmunoassay using methods described elsewhere in detail[17,18].

CATECHOLAMINE STUDY

Epinephrine and norepinephrine were determined according to the method of Da Prada and Zurcher[19], as modified by Michelet *et al.*[20].

STATISTICAL ANALYSIS

Paired t-tests were used to compare resting and smoking values.

Results

PLATELET STUDY

Plasma TXB_2 and βTG concentrations before and after smoking are shown in Fig. 1. The mean (±SD) plasma TXB_2 level was 118±40 pg per

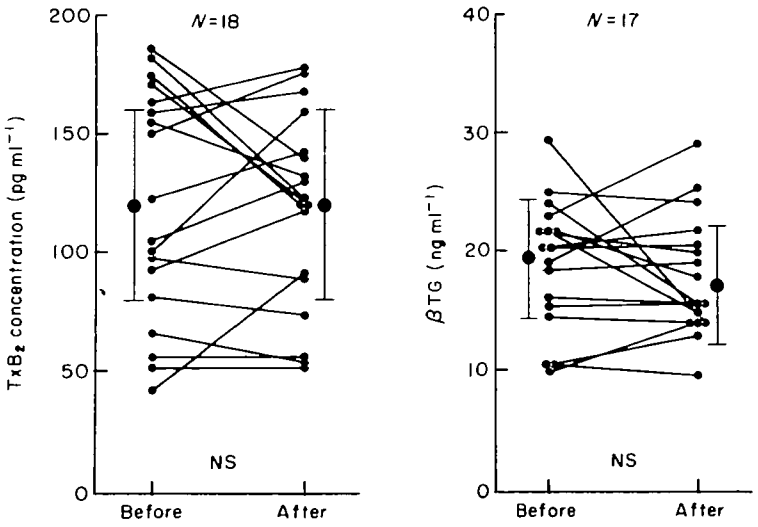

Figure 1 Platelet response to tobacco smoke.

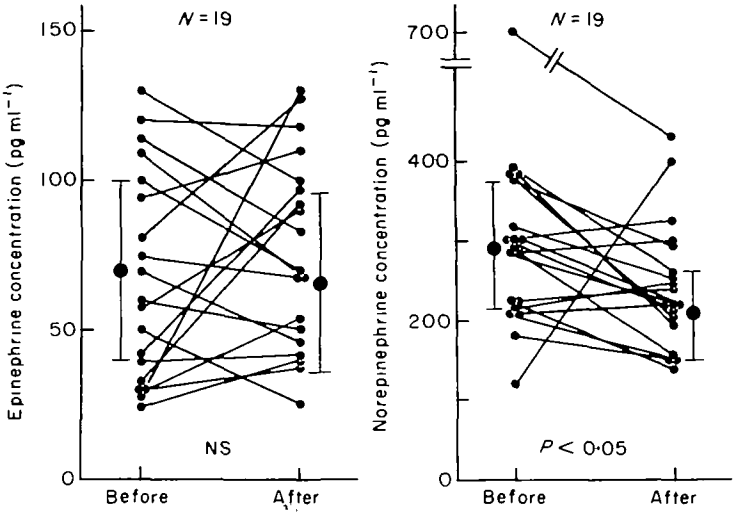

Figure 2 Changes in plasma epinephrine and norepinephrine during smoking.

milliliter before smoking, and did not change significantly 20 min after the beginning of smoking: 119 ± 41 pg ml^{-1}. The mean plasma βTG levels showed no significant change: 19 ± 5 ng ml^{-1} before and 17 ± 4 ng ml^{-1} after smoking.

CATECHOLAMINE STUDY

Figure 2 shows the individual and mean values ($\pm$SD) of plasma concentrations of epinephrine and norepinephrine before and 20 min after the beginning of smoking. We found no difference for epinephrine: 69 ± 30 pg ml^{-1} before versus 65 ± 26 pg ml^{-1} after, whereas we found a slight but significant decrease of norephinephrine: 288 ± 69 pg ml^{-1} versus 211 ± 48 pg ml^{-1} ($P < 0{\cdot}05$). However, there was a rather wide variation in the individual values.

In 5 subjects, blood samples were drawn every 5 min, in addition to non-invasive haemodynamic determinations.

The mean pulse rate, systolic blood pressure, double product and plasma epinephrine and norepinephrine concentrations before, during and after smoking are shown in Fig. 3. Smoking was associated with large increments of pulse rate, blood pressure and double product ($P < 0{\cdot}005$), but a significant fall of plasma norepinephrine concentration was observed, confirming the previous measurements. The epinephrine profile did not show any significant rise compared to the level prior to smoking; on the contrary, after 25 min, there was a significant fall of epinephrine.

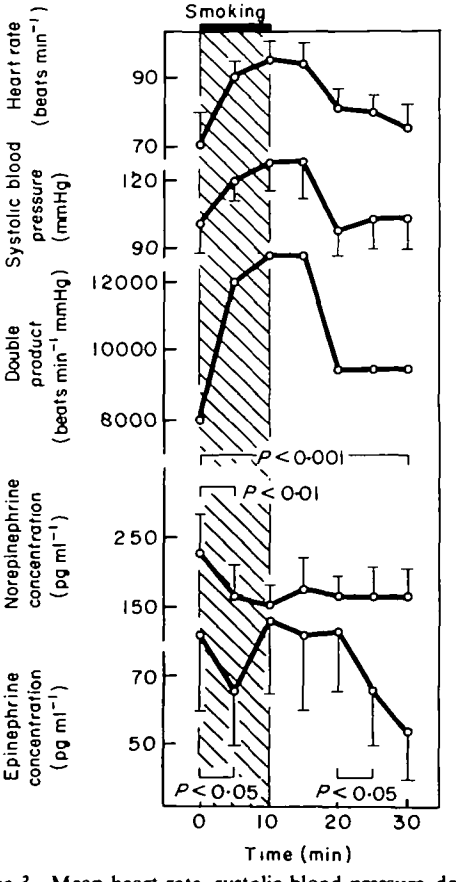

Figure 3 Mean heart rate, systolic blood pressure, double product and plasma catecholamines before, during and after smoking in five subjects.

NEUROPHYSINS

Figure 4 indicates the individual values of ESN before and after smoking. No change occurred: 353 ± 94 pg ml^{-1} versus 326 ± 111 pg ml^{-1}. However, as expected, the values of the females taking oral contraceptives were elevated, before and after smoking, being 2 to 4 times higher than the rest of the population.

Contrary to the effect on ESN, smoking induced a significant increase of NSN (see Fig. 5): 283 ± 52 pg ml^{-1} versus 520 ± 405 pg ml^{-1} ($P < 0{\cdot}05$). This increase did not occur in all the subjects. If we define 'responders' as subjects who increase their NSN levels more than 2 SD of the mean level prior to smoking, we find that only 12 out of the 22 subjects are 'responders'. The NSN increase was unrelated, in our population, to sex or to the platelet and catecholamine responses to smoking. Moreover, of the 5 subjects in whom haemodynamic measurements were recorded, only one was a 'NSN responder' during smoking, and had an haemodynamic profile similar to the other four.

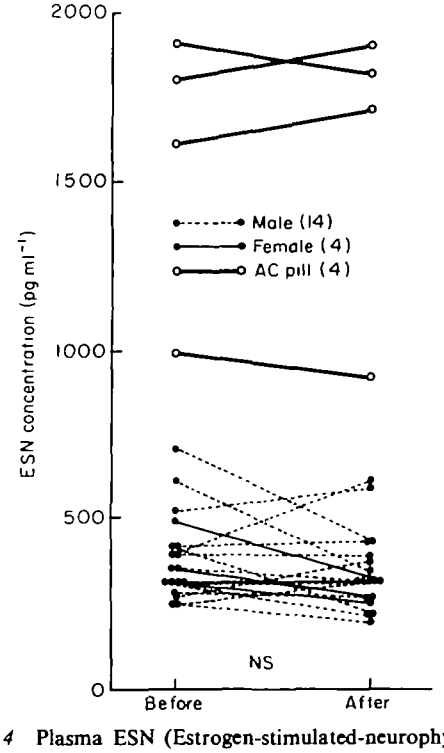

Figure 4 Plasma ESN (Estrogen-stimulated-neurophysin) before and after smoking. (AC pill: anticonceptional pill).

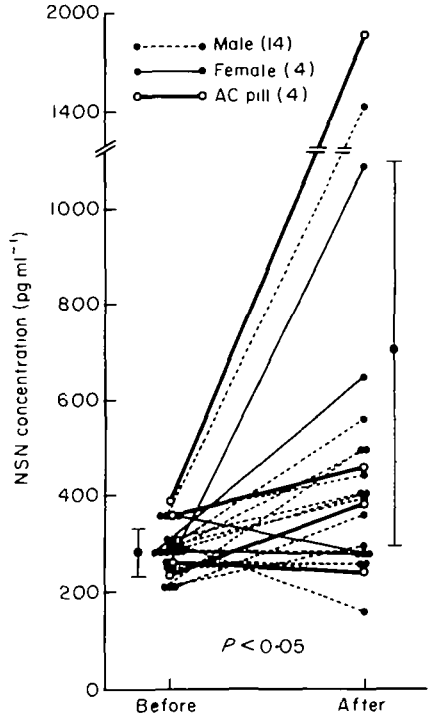

Figure 5 Changes of plasma NSN (nicotine-stimulated-neurophysin) before and after smoking.

Discussion

Our results show that in our population and during normal daily activity, cigarette smoking did not induce platelet activation, thromboxane formation, catecholamine rise or ESN secretion. However, the vasopressin-associated-neurophysin was significantly increased after smoking. This increase, which reflects AVP increase[8,16], occurred only in about half of our subjects, suggesting an individual susceptibility to cigarette smoke (and probably to nicotine).

In view of the putative triggering effect of smoke on acute coronary events, these observations are very important. It is well known that vasopressin administered therapeutically for gastrointestinal bleeding or for diabetes insipidus (even at low doses) can cause ischaemic accidents and sometimes sudden death in patients with coronary atherosclerosis[21]. As suggested by recent publications, vasopressin could act by promoting coronary spasm[22] or by elevating coronary resistance in patients with coronary artery disease[23,24].

In a previous study[4], smoking-associated

sympathetic discharge has been proposed as a mechanism for the adverse effect of smoking in patients with coronary atherosclerosis. There was no temporal association between the increase of pulse rate and blood pressure and the increase of plasma catecholamines. The authors proposed, however, that local catecholamine release mediated haemodynamic changes. In our study, despite a different protocol, smoking-associated increases in heart rate, blood pressure and subsequent double product were also found, but were not associated with an elevation of circulating catecholamines. Moreover, the catecholamine levels observed in our subjects are similar to those obtained in several studies of normal subjects in the sitting position[25]. Our study protocol was designed to maintain the usual conditions of the smoker in daily life and to avoid any additional experimental stress but the venipuncture. The haemodynamic changes cannot be attributed to the stress of the venipuncture since it began largely after the venipuncture (see Fig. 3). If these haemodynamic changes explain the occurrence of ischaemic accidents in subjects with an already compromised coronary circulation, via increases in cardiac work and oxygen consumption, they cannot be attributed to sympathetic discharge alone but to a more complicated mechanism. One hypothesis would involve an imbalance of the sympathetic and parasympathetic equilibrium, induced by nicotine effects at autonomic ganglia; indeed, the complex and often unpredictable changes that occur in the body after the parenteral administration of nicotine are partly due to its stimulant and depressant phases of action[26]. The minimal variations in norepinephrine and epinephrine levels measured in individual subjects after smoking suggest that the physical and psychological status of the smoker may play an important role and may attenuate the catecholamine changes observed in other reports.

The present work was specially designed to respect the normal conditions of daily activity. The differences in the observed findings between this and previous studies[5–7] could be due in part to the differences in the study protocol. For instance, in Cryer's study[5] the subjects were in the supine position for 30 min before and throughout smoking, while our subjects were not. This could explain higher catecholamine mean values before smoking in our total population and the absence of catecholamine increase (mean values) 20 min following smoking.

Finally, the haemodynamic changes observed in our study in the absence of catecholamine increase could be related to substances other than nicotine, inhaled during smoking and acting on the cardiovascular system, as suggested by recent publications[27–29].

Smoking-induced potentiation of platelet aggregation is another mechanism proposed to explain the increased incidence of ischaemic accidents in cigarette smokers[5]. Using sensitive methods for the in vivo detection of platelet activation, we cannot confirm this hypothesis in our population at 20 min following the beginning of smoking. These results are in accordance with a recent work in which blood was sampled in coronary sinus and studied with same methods[30]. However, our results do not exclude slight platelet modifications as previously demonstrated by in vitro provocative test[4].

Finally, oxytocin-associated-neurophysin is found to be extremely high in the 4 females who were taking oral contraceptives, and was not related to smoking. We have previously shown, in a larger population, high values of ESN in women on oral contraceptives, during pregnancy and in men undergoing diethylstilbestrol therapy for prostate carcinoma[17]. Such high ESN levels probably reflect very high levels of oxytocin which can promote cardiac accidents in predisposed subjects[31]. Although the vasoactive properties[32] of oxytocin are not well defined (particularly in women during chronic use), this finding could bring a new light on the fact that oral contraception increases the risk of cardiovascular morbidity and mortality[33]. Further and larger studies are necessary to confirm this hypothesis.

In conclusion, it was recently demonstrated that cessation of smoking after myocardial infarction is associated with a considerably higher survival rate and lower cumulative frequency of reinfarction[12]. The present study suggests that this beneficial effect could be the result of improvement of a factor such as vasopressin secretion rather than platelet activation or catecholamines release.

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