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Cigarette smoke and hypoxia induce acute changes in the testicular and cerebral microcirculation

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ABSTRACT

The acute effects of cigarette smoking and hypoxia on the cerebral and testicular microcirculation were studied in anestethised adult rats. Smoking for 2 min did not influence arterial pO_2, pCO_2 or pH but it induced an increase in cerebral blood flow by 34% and inhibited vasomotion in the testis for about 1 h. One hour after smoke exposure apnea induced a slight increase in arterial pCO_2, a significant decrease in pO_2, and an increase in cerebral blood flow (CBF) by 54%. In animals not previously exposed to cigarette smoke apnea increased CBF by 121%, demonstrating that a short-term exposure to tobacco smoke influences the cerebrovascular reactivity for more than one hour. In the testis, apnea resulted in a decreased blood flow by 39% and a complete depression of vasomotion. Breathing 10% O_2 / 90% N_2 resulted in moderate hypoxia, a total disappearance of the vasomotion in the testis, a 24% decrease in testicular blood flow, but a 23% increase in CBF.

Our results indicate that short- term exposure to tobacco smoke induces marked acute vascular effects in both the brain and the testis. Apnea and moderate hypoxia elicited totally different effects in the brain and testis, indicating different vascular control mechanisms.

INTRODUCTION

Tobacco smoking is a risk factor for the development of several cardiovascular and malignant diseases but the mechanisms involved are not fully understood. Smoking has also been suggested to reduce male fertility. Particularly when acting in synergy with other fertility reducing factors like varicocele, it may cause decreases in sperm count, sperm motility (16,17,25) and testosterone secretion (29). The pathogenic mechanisms behind this is, however unknown. We recently observed that exposure to cigarette smoke directly influenced testicular blood flow. The testicular microcirculation is characterised by prominent rhythmical variations in capillary blood flow (vasomotion) and vasomotion is directly inhibited by tobacco smoke. As vasomotion is

involved in transvascular fluid exchange in the testis (4) we suggested that this disturbance could be involved in the pathogenesis of smoke-induced infertility (10).

In this study we wanted to explore further the mechanism by which acute smoke exposure affects testicular blood vessels and in particular examine whether these changes are related to changes in blood gases. For this purpose, arterial pO_2, pCO_2 and pH were analyzed during smoke exposure. The microcirculatory response to tobacco smoke exposure was also compared to that induced by apnea and moderate hypoxia. The response in the testis was compared to that in the brain. The brain is an organ particularly susceptible to hypoxia and control mechanism therefore operating to maintain tissue perfusion and oxygen delivery during general hypotension and hypoxia, but such mechanism may not be present in the testicular microcirculation (4,22). The effects of tobacco smoke exposure on the cerebral microcirculation are not fully explored. Cigarette smoking is a risk factor for hemorrhagic and non-hemorrhagic stroke in man (11,15,19) and cerebral vasospasm caused by subarachnoid hemorrhage is increased in smokers (21). Some of the tobacco-induced long-term effects could be due to effects mediated by alterations in the microcirculation in the brain. However, contradictory effects of tobacco smoking and nicotine on cerebral blood flow have been reported (20,32,33,36).

METHODS

Male Sprague-Dawley rats (Møllegaard, Ejby, Denmark) weighing 363-377 g were used. Animals were housed 5 rats per cage. The room temperature was 22 ± 2 °C and the humidity 50 ± 5%. The room was artificially lightend in a 12 hr light/dark cycle. The animals had free access to the pellet R34 diet (Lactamin, Vadstena, Sweden) and tap water. All experiments were performed between 8 am and 2 pm. The experiments were approved by the Regional Research Ethical Committee according to the national law.

Anaesthesia was induced by injecting 120 mg kg^{-1} thiobutabarbital (Inactin®, RBI, Natick, MA, USA) intraperitoneally. The animal was tracheostomized and a small animal ventilator (model 683, Harvard Apparatus, South Natick, MA, USA) connected to the tracheal line. Skeletal muscle relaxation was induced by pancuronium bromide (Pavulon ®, Organon, Oss, Holland) in a dose of 0.2 mg kg^{-1} intravenously (i.v.). Both femoral arteries were cannulated. One was used for continuous mean arterial blood pressure (MAP) monitoring with a Gould Statham P 23 ID pressure transducer (Gould Inc, Oxnard, Ca, USA) and a SE 120 recorder (ABB- Goerz-Metrawatt, Vienna, Austria). The other artery was used for blood sampling. Arterial PO_2, PCO_2 and pH were determined at intervals with an ABL 500 acid-base analyzer (Radiometer, Copenhagen, Denmark). One femoral vein was cannulated and used for continuous infusion of

0.5 ml h^{-1} 100g body wt^{-1} Ringer solution (25mM $NaHCO_3$, 120mM NaCl, 2.5mM KCl, 0.75mM $CaCl_2$) provided by a syringe pump (341A, SAGE Instruments, Cambridge, MA, USA) in order to substitute for the normal fluid loss. The rectal temperature was continuously measured and maintained at 37°C by a CMA 150 servo heating pad (Carnegie Medicin, Stockholm, Sweden).

The shaved skin of the head and the outer ear meatuses were pre-treated with lidocain hydrochloride (Xylocain® viscous, Astra, Södertälje, Sweden). The animal was placed in a stereotaxic frame (I.H. Wells Jr., Mechanical Developments Co., South Gate, CA, USA). The parietal bone was exposed and a small burr hole of 2-3 mm in diameter was made using a drill with a flat bottom. The centre of the hole was 3 mm caudal to the coronal suture and 3 mm lateral to the sagittal suture. Continuous cooling with physiological saline was performed while drilling. Care was taken to leave a thin bone layer intact (0.1 mm) in order not to disturb the cortical blood flow. Dural and pial blood vessels were readily recognised through the partial craniotomy.

Cortical microcirculation (CBF) was measured by the laser-Doppler flowmeter Periflux PF4000 (Perimed, Järfälla, Sweden). Using a micromanipulator, the measuring probe (PF 403, outer diameter 1.0 mm, fiber diameter 0.125 mm, fiber separation 0.25 mm) was positioned on the thin bone layer. Large dural and pial vessels were avoided. The probe measures the blood flow within a hemisphere with a diameter of about 1 mm (5) and thus the above preparation procedure is sufficient to enable measurement of local cortical cerebral blood flow (12,27). However, under some conditions the method can overestimate an increase in blood flow (13).

The testis was exposed via a scrotal incision. A PF 412 multireceiver probe (Perimed, Järfälla, Sweden) with 7 receiver fibres separated from the sending fibre by 1.2 mm was used for the measurement of blood flow in the testis (TBF). This probe allows a wider and deeper measurement range compared to standard probes.

At least 30 min elapsed from the positioning of the probes to the start of blood flow recordings. The laser-Doppler signal was transferred on-line to a PC and analysed with Perisoft software (Perimed). The flowmeter was calibrated by measuring the relative flow value in a test vial containing a colloidal suspension of microscopic latex particles in random Brownian motion. The regional vascular resistance (R), reported as vascular resistance units (PRU) was calculated as $R=MAP \times Q_t^{-1}$ where Q_t is tissue blood flow.

The exposed animals (n=7) were connected to a smoking device via the ventilator. The smoking device consists of two timer controlled pumps, one delivering cigarette smoke and one fresh air (10). The animals were repeatedly exposed to cigarette smoke for 2 sec and fresh air for 2 sec during a total test period of 2 min. During the smoking period the laser-Doppler signals were averaged over several periods during the maximal response. Standard cigarettes containing

0.9 mg nicotine and 10 mg tar were used. After the cigarette smoking period, the animals were allowed to breathe fresh air via the ventilator. Prior to finishing the experiment, the ventilator was stopped for 30 sec (apnea test) and the blood flow change was measured. This was done in order to study whether the organs respond differently to the stress of apnea including the decrease in arterial PO_2 and increase in PCO_2. A second aim of the apnea test was to make sure the proper function of the laser-Doppler probe. At the end of the experiment, the animals were sacrificed by a lethal i.v. dose of pentobarbital (Apoteksbolaget, Umeå, Sweden). A schematic picture of the experimental protocol is shown in figure 1.

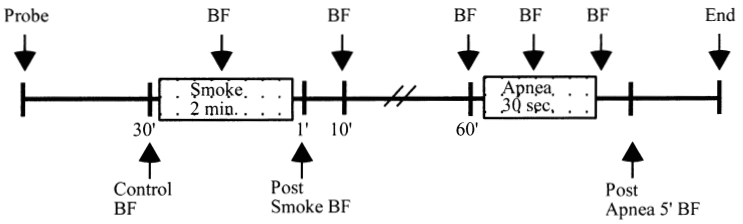

Fig. 1. Schematic depiction of the experimental protocol. BF=blood flow. Figures followed by ' are in min.

In the second series of experiments the animals (n=6) were prepared as described above excluding the smoking procedure. After a control period, the animals were exposed for 5 min to a gas mixture of 10% O_2/90% N_2 connected to the inlet of the respirator in order to produce a mild hypoxia. At the end of hypoxia the ventilator was switched to atmospheric air. Thirty min later apnea was induced as described above.

Statistical analysis of the results was performed with analysis of variance (ANOVA) followed by t-test with Bonferroni correction when appropriate. The statistical comparison of the effect of apnea on the microcirculation in the hypoxia group and the smoke-exposed group was done with unpaired Student's t-test. Results are reported as means ± S.E.M.

RESULTS

Cardiovascular and blood gas parameters

The MAP was stable during the smoking experiment (table 1). However, a statistically significant decrease by 28 % ($p<0.001$) was observed at the end of the apnea test. MAP returned to a normal level within 1 min after the apnea (table 1). Arterial blood gases and pH were not significantly changed during the exposure to cigarette smoke or fresh air (table 1). Arresting the

ventilator for 30 sec induced a major decrease in arterial PO_2 and concomitantly there was an increase in PCO_2 and a decrease in pH (table 1).

As described in table 1, MAP was significantly affected at the end of the hypoxia and normalised after the hypoxia. The arterial PO_2 was decreased from 13.09 ± 0.37 kPa to 9.47 ± 0.41 kPa during the hypoxia and returned to control level after hypoxia. Apnea caused a similar effect as in the first experimental series (table 1).

Table 1 Cardiovascular parameters and blood gas values

	MAP (kPa)	**pH_a**	**P_aO_2 (kPa)**	**P_aCO_2 (kPa)**
Control 1	16.1±1.1	7.40±0.01	12.52±0.33	4.72±0.19
Smoke	16.6±1.6	7.38±0.02	12.60±0.56	5.44±0.03
Post Smoke	15.0±2.7	7.36±0.01	12.29±0.87	5.02±0.40
Pre Apnea	17.4±2.7	7.37±0.01	12.75±0.49	5.18±0.26
Apnea	12.6±3.0***	7.32±0.01***	4.49±0.76**	5.84±0.44
Control 2	17.9±0.7	7.47±0.01	13.09±0.37	4.76±0.10
Hypoxia	9.8±0.6##	7.45±0.01	9.47±0.41#	4.63±0.08
Post Hypoxia	18.8±0.3	7.48±0.01	13.29±0.41	4.50±0.12
Pre Apnea	18.8±0.3	7.48±0.01	13.29±0.41	4.50±0.12
Apnea	12.9±0.9**	7.45±0.01	11.78±0.74*	4.88±0.14**

Control 1 is before smoking (smoke), post-smoke is 10 min after smoking and pre apnea is during baseline situation before apnea. Control 2 is before 5 min of hypoxia (hypoxia), post hypoxia is 10 min after hypoxia and pre apnea is during baseline situation before apnea. *$p<0.05$, **$p<0.01$, ***$p<0.001$ as compared to pre apnea and #$p<0.01$, ##$p<0.001$ as compared to control.

Testicular microcirculation

Smoke exposure and apnea test

Testicular blood flow was slightly increased (5.7 ± 2.8%, $p = 0.09$) by the cigarette smoke (Fig.2A). The baseline vascular resistance in the testis was 0.073 ± 0.007 PRU and slightly decreased to 0.071 ± 0.009 PRU during the smoking. The cigarette smoke markedly affected the frequency in vasomotion and the amplitude of vasomotion. The baseline frequency was 0.15 ± 0.01 Hz and initially increased to 0.16 ± 0.01 Hz during the smoke exposure but at the end of the exposure period, vasomotion totally disappeared. The amplitude was 184.3 ± 24.1 and decreased to 107.0 ± 29.7 PU during the smoking ($p<0.001$) and further reduced to 53.6 ± 8.7 ($p<0.001$) at the end of smoking. Ten min later, the vasomotion frequency was still reduced to a value of 0.06 ± 0.01 Hz ($p<0.05$) and the amplitude to 128.9 ± 36.4 (not significant). The frequency only slowly returned to the baseline level. Fifty min after the cigarette smoke exposure, the vasomotion frequency was 0.12 ± 0.01 Hz ($p<0.05$). Before the apnea test (1 h after smoke exposure), the vasomotion frequency (0.13 ± 0.03 Hz) and amplitude (167.6 ± 31.8 PU) were normalised.

During the period of apnea, the vasomotion totally disappeared and the amplitude decreased to 31.2 ± 3.4 PU ($p<0.001$) as compared to pre apnea. A few min after the apnea experiment, vasomotion reappeared and the frequency was 0.13 ± 0.03 Hz 5 min after the end of apnea and the amplitude was 225.2 ± 21.3 PU. Apnea induced a statistically significant decrease in testicular blood flow by 39.1 ± 7.8 % ($p<0.001$) (Fig. 2B) concomitantly with an increase in vascular resistance to 0.120 ± 0.027 PRU ($p<0.01$) as compared with pre apnea values. The blood flow returned to control level rapidly (Fig. 2B) without any prominent reactive hyperemia. The testicular blood flow was 107.0 ± 8.2 % as compared to the blood flow before apnea.

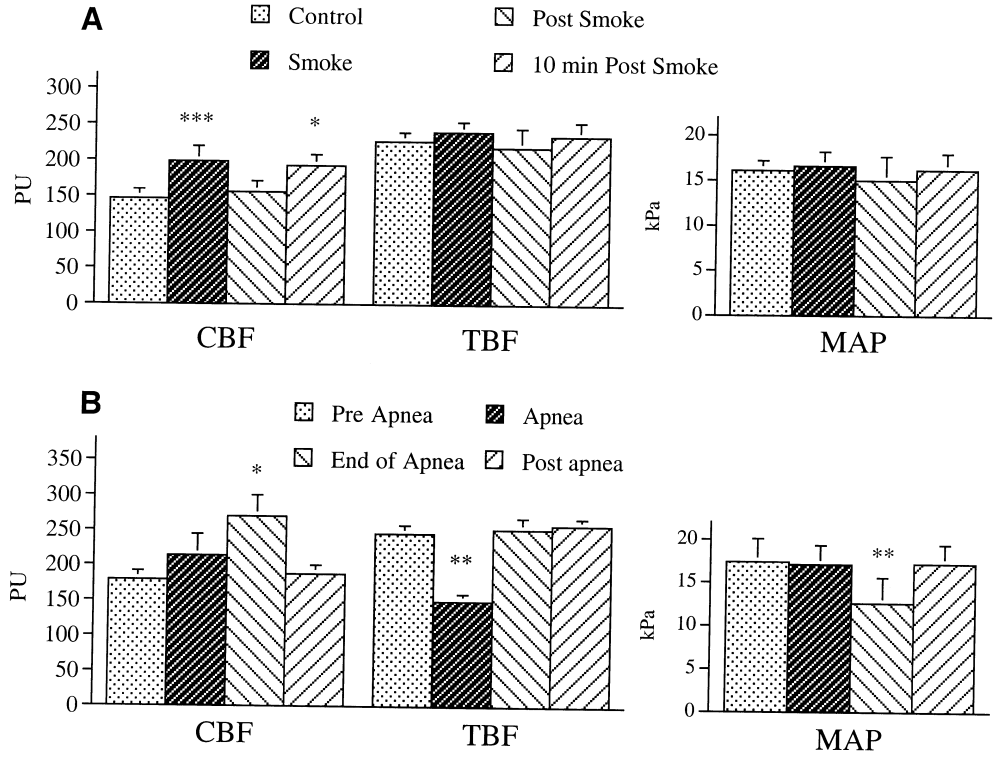

Fig. 2A and 2B. Mean arterial blood pressure (MAP), cerebral cortical (CBF) and testicular (TBF) blood flows in control situation (control), during smoking (smoke), immediately after smoking (post smoke), 10 min after smoking, before apnea (pre apnea), at 10 sec of apnea (apnea), end of apnea, and 5 min after apnea (post apnea). Blood flows are expressed as perfusion units (PU). *$p<0.01$, **$p<0.005$, ***$p<0.002$ as compared with control.

Moderate hypoxia and apnea test

Breathing 10% O_2 induced a moderate hypoxia (Table 1) and this resulted in a similar microvascular response as apnea. Thus the vasomotion, with a control frequency of 0.12 ± 0.01

Hz totally disappeared already after 32 ± 4 sec and reappeared when the PO_2 was normalised. Control TBF was 182.5 ± 20.7 PU and decreased to 135.9 ± 17.7 PU ($P<0.05$) when the vasomotion was decreased. This corresponds to a decrease in flow by 24.4 ± 6.1% ($p<0.0001$). At 5 min hypoxia the TBF further decreased to 79.6 ± 16.4 PU ($P<0.01$) representing a decrease by 54.0 ± 10.0% ($p<0.005$). The corresponding values for vascular resistance were 0.106 ± 0.015 PRU, 0.137 ± 0.014 PRU ($P<0.05$) and 0.155 ± 0.034 PRU ($P>0.05$), respectively. Thirty min later an apnea test was performed. As in the animals exposed to cigarette smoke apnea inhibited testicular vasomotion. The frequency decreased from 0.13 ± 0.01 Hz to zero. TBF decreased from 203.8 ± 22.6 PU to 178.0 ± 20.5 PU ($p<0.05$) representing a decrease of 15.1 ± 2.8 %. The vascular resistance increased from 0.100 ± 0.014 PRU to 0.112 ± 0.016 PRU ($p<0.05$).

Cerebral microcirculation

Exposure to cigarette smoke directly increased CBF by 34.1 ± 5.5 % ($p<0.001$) (Fig. 2A) and decreased vascular resistance from 0.112 ± 0.007 PRU to 0.087 ± 0.008 ($p<0.001$). CBF returned to control level immediately after the cigarette smoke exposure period. However, about 10 min later the CBF was once again increased by 34.3 ± 9.4 % ($p<0.01$) and vascular resistance decreased to 0.079 ± 0.013 PRU ($p<0.05$). This cortical hyperemia was long lasting and gradually decreased toward the control level. One hour after the smoke exposure the CBF was still somewhat elevated to 178.6 ± 17.3 PU as depicted in figure 2B as preapnea value. At this point, the apnea test induced an increase in CBF by a maximal value of 54.0 ± 14.5% ($p<0.01$) and a decrease in vascular resistance to 0.045 ± 0.012 PRU ($p<0.005$). CBF was normalised 5 min after the apnea (Fig. 2B).

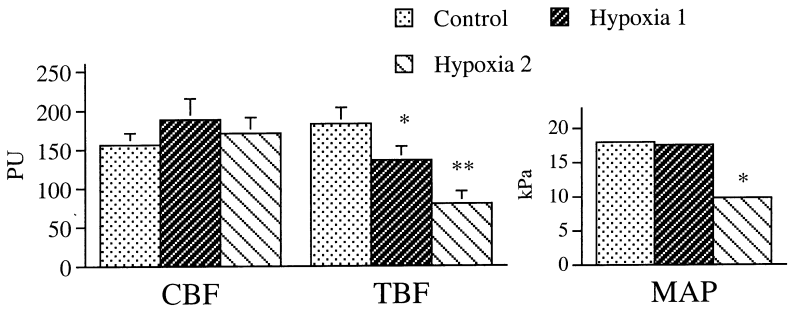

Fig.3. Mean arterial blood pressure (MAP), cerebral cortical (CBF) and testicular (TBF) blood flows in control situation (control), at 30 sec (hypoxia 1) and 5 min of hypoxia (hypoxia 2). *$p<0.05$, **$p<0.01$ as compared to control.

Hypoxia induced a rapid increase in CBF by 23.5 ± 20.9% ($p<0.05$) and after 5 min of hypoxia the increase was 10.1 ± 0.9% ($p<0.0001$) (Fig 3). This effect represents a vasodilation as the vascular resistance decreased from 0.120 ± 0.012 PRU to 0.104 ± 0.017 PRU and further to 0.061 ± 0.008 PRU ($p<0.001$) after 5 min of hypoxia. In these animals apnea induced a 121.1 ± 34.2% ($p<0.05$) increase in CBF and a 51.1 ± 7.9 % ($p<0.001$) decrease in vascular resistance. Thus, the effect of apnea on CBF was decreased in tobacco smoke-exposed animals ($p<0.05$, unpaired Student's t-test).

DISCUSSION

The testicular microcirculation is characterised by a prominent, regular and high amplitude vasomotion (4). The functional role of testicular vasomotion is unknown, but indirect evidence suggests that vasomotion may be particularly important in the testis as a way to promote interstitial fluid resorption back to the circulation at the venous side of the microvasculature (4). The factors regulating testicular vasomotion frequency and amplitude are largely unknown but testosterone and other locally produced factors are involved (4,8,9). Our previous observation that exposure to tobacco smoke directly inhibits testicular vasomotion is therefore of some interest as a way to explore the regulation of testicular vasomotion and the functional consequences of disturbances in this. In our previous experiment, however, we did not monitor blood gases, pH and blood pressure during and after smoke exposure. It was therefore possible that smoke exposure has effects on these parameters and that testicular microcirculation is particularly susceptible to changes in for instance pO_2 and not to tobacco smoke per se. The present study confirms our previous observation (10) that short-term exposure to cigarette smoke inhibits vasomotion in the testicular microcirculation and demonstrates that this is not due to changes in blood gases or pH. The active factor(s) in tobacco smoke and its mode of action however remains unknown. In our previous study, testicular blood flow was moderately but significantly increased during smoke exposure (10) but this was not the case in the present study. This difference could be related to the differences in smoke dose, ventilator vs. spontaneous breathing, or to differences in anaesthesia and muscle relaxation in the two experiments.

Interestingly, apnea induced a rapid decrease in testicular blood flow and increase in vascular resistance. Concomitantly, the vasomotion totally disappeared. During the apnea, the PO_2 and blood pressure decreased and PCO_2 increased. Previous studies have suggested that the testicular circulation lacks autoregulation and that blood flow is directly related to blood pressure (14,22). The reduced flow could thus be related to the drop in blood pressure, but the increased vascular resistance and the inhibited vasomotion suggest local adaptations within the testis and indicate that the testicular microcirculation could be sensitive to changes in blood pressure or in the

partial pressures of oxygen and carbon dioxide in the blood. In other organs like brain and muscle, vasomotion does not disappear unless blood pressure is less than 50% of normal (26,27). Interestingly, a relative mild hypoxia totally abolished the testicular vasomotion and decreased testicular blood flow. Thus, the partial pressure of oxygen is at least one of the mechanisms regulating the testicular vasomotion and blood flow.

In the present study we demonstrate that the testicular microcirculation is highly sensitive to moderate hypoxia, but it responds to it with a decrease in blood flow, an increase in vascular resistance and inhibition of vasomotion. This response is the opposite to that in the brain where threatening tissue hypoxia is apparently met by an increase in blood flow and a decreased vascular resistance. Previous studies have shown that testicular blood flow is directly reduced when blood pressure decreases (22), i.e. the testis is unable to autoregulate its blood flow. In addition to this, we now demonstrate that moderate systemic hypoxia results in a decrease in testicular blood flow. The testis apparently lacks control systems that in other tissues such as the brain maintain tissue perfusion and oxygen delivery during situations with moderate hypotension and hypoxia. In the spermatogenic epithelium millions of sperms are produced every day and this obviously requires a continuous supply of oxygen and nutrients. The oxygen tension in the seminiferous tubules is already under normal condition remarkably low (18). Sclerotic changes in the testicular artery are not uncommon in both young and elderly men (6,28). It can therefore be hypothesised that factors that reduce testicular blood flow or disturb its microcirculation could result in decreased sperm production. We have previously suggested that vascular disturbances could be an important but generally ignored factor in the pathogenesis of male infertility. The present demonstration that the testicular microcirculation is sensitive to tobacco smoke inhalation and that it acutely responds to moderate hypoxia with a decrease in blood flow support this hypothesis.

Tobacco smoke has been reported to have no effect on cerebral blood flow (CBF), oxygen consumption, vascular resistance or blood gases in the human (36). Solti and co-workers (34) reported no significant changes in CBF after smoking or injection of 1 mg nicotine. However, in more recent investigations, contradictory results are reported. Thus, tobacco smoking and intravenous infusion of nicotine in man elicit an increase in CBF without a significant effect on the cerebral metabolic rate of oxygen in the brain (33). Interestingly, it was recently reported that CBF, measured by the pulsed Doppler method, was increased in newborn infants of smoking mothers (1). Thus, it seems likely that tobacco smoke elicits vascular effects also in systems protected by blood barriers, in this case by the blood placenta barrier and the blood brain barrier in the neonatal. An increase in the mean flow velocity in the middle cerebral artery of smoking adults (32) and a decrease in CBF in chronic smokers as compared to non-smokers has been reported (20,30,31). Furthermore, there is little doubt that tobacco smoking is a risk factor for

stroke (11,15,19), for review see (2). Interestingly, it was recently reported that tobacco smokers have an increased risk in developing cerebral vasospasm as a consequence of subarachnoid hemorrhage (21). From the above mentioned studies in humans, it seems clear that tobacco smoking affects the cerebral blood flow.

In our study, we clearly demonstrate that acute tobacco smoking increases the cerebral blood flow in the rat. It is unlikely that alterations in blood gases or blood pressure mediates this effect. Indeed, it has been shown that infusion of nicotine elicits an increase in CBF in animals (3). Effects of acute and chronic administration of nicotine on cerebral metabolism have also been shown in the rat (23,24). A part of the cerebrovascular effect of nicotine could be explained by an effect mediated by the autonomic nervous system involved in the chemoreceptor response (35). Interestingly, it was recently shown in the rat that nicotine administered sc. raises the influx of permeable solutes across the blood-brain barrier concomitantly with an increase in the local cerebral blood flow (7). The cerebrovasodilating effect of tobacco smoke shown in our study could thus be elicited by nicotine. However, as tobacco smoke consists of a variety of compounds, it is impossible to exclude these as contributors to the cerebrovascular effect.

The well established effect of acute hypoxia and/or hypercarbia on the cerebral circulation was demonstrated by the apnea test. The cerebral blood flow increased immediately and returned to control level soon after the apnea test. This shows that our experimental setup was able to accurately measure cerebral blood flow. Hypoxia *per se* caused a cerebral vasodilation. It has recently been shown that cigarette smoking in the human decreases the cerebrovascular reactivity to apnea (32). Interestingly, our results also show a difference in cerebrovascular reactivity in the same manner as reported by Silvestrini and co-workers (32). However, in our study the difference was not statistically significant.

In conclusion, our results show that smoking affects the cerebral and testicular microcirculation. The cerebrovascular reactivity tended to be affected by smoking. One can speculate that repeated exposures affect the regulation of the cerebral blood flow. This can be one of the reasons for the negative effects of smoking on cerebral hemodynamics. A totally different effect was elicited in the testis showing different control mechanisms in these two organs. Hypoxia abolished the vasomotion in the testis. Thus, hypoxia in the apnea test probably was the cause of the effect on testicular vasomotion.

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