Psychopharmacology (1990) 102:68–72

Psychopharmacology

Smoking-associated changes in the serotonergic systems of discrete regions of human brain

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Received July 13, 1989 / Final version March 10, 1990

Abstract. This paper describes the results of a postmortem study of the effects of tobacco smoking on the concentrations of 5-hydroxytryptamine (5–HT) and 5-hydroxyindoleacetic acid (5–HIAA) as well as the binding of [^{3}H]-8-hydroxy-(di-n-propylamino)-tetralin ([^{3}H]-8-OH-DPAT) and [^{3}H]-ketanserin in six discrete regions of human brain. Smoking was associated with significant decreases in the concentrations of 5–HIAA in the hippocampal neocortex ($P<0.001$), hippocampal formation ($P<0.05$) and the median raphe nuclei ($P<0.05$). The 5–HT level of the hippocampal formation was also significantly reduced in smokers ($P<0.05$). These changes were accompanied by significant increases in the binding of [^{3}H]-8-OH-DPAT in the hippocampal neocortex ($P<0.01$) and hippocampal formation ($P<0.05$). [^{3}H]-Ketanserin binding in the brain regions studied was unaffected by smoking. It is concluded that smoking is associated with a regionally selective decrease in the activity of the serotonergic system of the human hippocampus.

Key words: Tobacco smoking – Human brain – 5-Hydroxytryptamine – 8-OH-DPAT binding – Ketanserin binding – Hippocampus

It is widely accepted that nicotine plays an important part in the tobacco smoking habit and that many smokers become dependent upon the nicotine present in the smoke (Gilbert 1979; Henningfield 1984). Little, however, is currently understood of the neurochemical mechanisms which mediate the development of nicotine dependence, although previous studies in this laboratory have shown that chronic nicotine administration to rats causes a regionally selective decrease in the concentration and biosynthesis of 5-hydroxytryptamine (5–HT) in the hippocampus (Benwell and Balfour 1979, 1982) and it has been suggested that these effects may be associated with the development of dependence upon the drug (Balfour 1982a, b). The purpose of the experiments reported in this paper was to examine further the possible role of hippocampal 5–HT systems in nicotine dependence by investigating the changes which occur in the hippocampus of people who have smoked cigarettes over a prolonged period of time.

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Materials and methods

Post-mortem/tissue. Details of sample collection are similar to those published previously (Benwell et al. 1988). Briefly, brain samples were obtained from 30 cadavers sent to the Department of Pathology at Ninewells Hospital and Medical School in Dundee for routine necropsy. Only subjects whose clinical notes contained clear information concerning their smoking habits were included in the study. Non-smokers were defined as those who had never smoked or who had given up smoking at least 5 years prior to death. For subjects falling into the latter category, the mean interval between stopping smoking and death was 18 ± 5 years. Each of the smokers had smoked cigarettes for at least 30 years and claimed to smoke between 7 and 20 cigarettes per day in the 2 years prior to death, although some had previously smoked more heavily. Subjects who were smokers were selected for the study when it could be established that they had continued to smoke to within 48 h of their death. Samples were obtained from 12 smokers [6 males and 6 females with a mean age at death of 74 ± 4 years (range 55–83)] and 18 non-smokers [7 males and 11 females with a mean age at death of 74 ± 3 years (range 60–88)]. The causes of death were ischaemic heart disease, bronchopneumonia or malignant neoplasms, the incidence of each cause of death being evenly distributed between the smokers and non-smokers. None of the subjects had shown any symptoms of psychotic illness and all had been free of neurological disorders. None of the brains sampled had gross abnormalities on sectioning, and in particular no evidence of ischaemic damage or neoplasm was found in the central nervous system. All brain samples were collected within 72 h of death, the mean postmortem delay (i.e. the time between death and freezing the tissue samples at $-70°$ C for storage) was 33 ± 7 h for non-smokers and 35 ± 9 h for smokers. Tissue was taken from two parts of the medial temporal lobe: (a) the hippocampal formation including Ammon's horn ± subiculum (HF) and (b) the hippocampal neocortex (HNC) (Brodmann area 27), from one region of the frontal lobe [gyrus rectus (GR) (Brodmann area 11)] and also from the cerebellar cortex (CC), the median raphe (MRN) of the midbrain and the caudal part of the medulla

oblongata (MO). The samples from the hippocampus and the frontal cortex were carefully dissected to remove all white matter. The other samples from the anatomically complex areas were not dissected. However, the cerebellar cortex and median raphe nuclei were chiefly grey matter and the medulla oblongata mainly white matter. Tissue samples were stored at −70° C until assayed.

Biochemical assays. The concentrations of 5–HT and 5-hydroxyindoleacetic acid (5–HIAA) in the brain samples were analysed using high performance liquid chromatography with electrochemical detection using the procedure of Reinhard et al. (1980). Tissue preparation and incubation procedures for the 5–HT receptor assays were those described by Middlemiss and Fozard (1983). The 5–HT_{1A} receptor subtype was labelled by [^{3}H]-8–hydroxy-(di-n-propylamino)-tetralin ([^{3}H]-8-OH-DPAT) (1 nM) and the 5–HT_2 receptors specifically labelled by [^{3}H]-ketanserin (1 nM). Non-specific binding was determined by incubating membranes with the radiolabelled ligands, in the presence of either 10 μM unlabelled 5–HT (for [^{3}H]-8-OH-DPAT) or 10 μM mianserin (for [^{3}H]-ketanserin). Radioligand binding to brain nicotinic receptors was measured using [^{3}H]-(-)-nicotine (14 nM) according to the procedure described in Benwell et al. (1988). Protein estimations were carried out using the method of Lowry et al. (1951).

Data analysis. Statistical analyses of the data were performed using the statistics package for social scientists (SPSS PC ± version). The data were analysed by two-way analysis of variance, with sex and smoking as the independent factors analysed and age and postmortem delay as covariates. The equilibrium dissociation constants (K_d) and densities (B_{max}) of receptor sites were determined statistically using microcomputer version of LIGAND, the curve fitting program of Munson and Rodbard (1980).

Drugs. Drugs used in the biochemical assays were 5–HT creatinine sulphate (Sigma), pargyline hydrochloride (Sigma), mianserin hydrochloride (Organon) and nicotine hydrogen tartrate (Aldrich). [^{3}H]-8-OH-DPAT (157.1 Ci/mmole), [^{3}H]-ketanserin (76.6 Ci/mmole) and (-)-[^{3}H]-nicotine (73.7 Ci/mmole) were purchased from New England Nuclear.

Results

Of the brain regions studied, the highest concentrations of 5–HT and 5–HIAA were found in the median raphe nuclei, the lowest being found in the cerebellar cortex and gyrus rectus (Fig. 1). Smoking was associated with significant reductions in the concentrations of 5–HT [F smoking (1,20) = 7.5; $P < 0.05$] and 5–HIAA [F smoking [1,22] = 4.7; $P < 0.05$] in the hippocampal formation and of 5–HIAA in the hippocampal neocortex [F smoking (1,24) = 16.1; $P < 0.001$] and the median raphe nuclei [F smoking (1,24) = 5.1; $P < 0.05$]. There was a small but significant F smoking (1,20) = 4.9; $P < 0.05$] increase in the 5–HT concentration of the gyrus rectus of smokers. However, in this region of the brain the sex of the subject influenced the effect of smoking [F smoking by sex (1,19) = 6.5; $P < 0.05$]. Subsequent analysis showed that smoking increased the concentration of 5–HT in the gyrus rectus of males from 0.026 ± 0.006 to 0.045 ± 0.015 [F smoking (1,8) = 5.5; $P < 0.05$], whereas smoking had no effect on the 5–HT levels in females.

Displaceable [^{3}H]-8-OH-DPAT binding was greatest in the hippocampal formation, hippocampal neocortex and gyrus rectus with lower levels found in the median

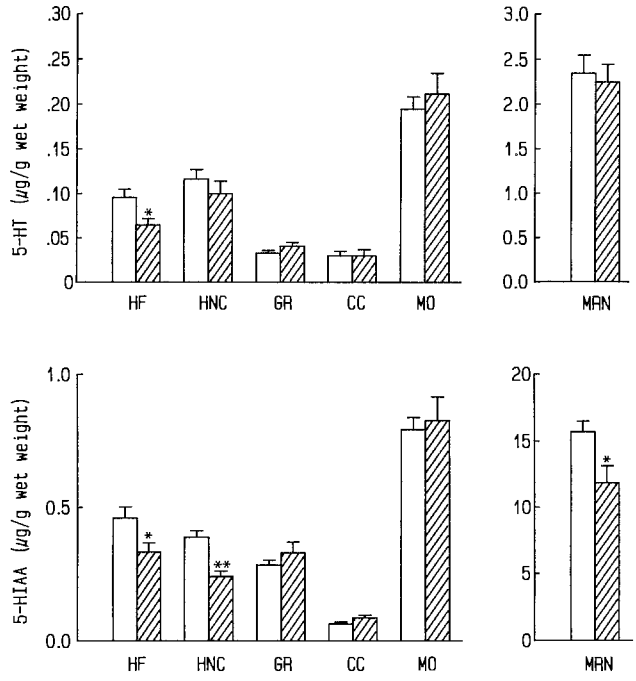

Fig. 1. The effect of smoking on the 5–HT and 5–HIAA concentrations in human brain. 5–HT and 5–HIAA concentrations in the hippocampal formation (*HF*), hippocampal neocortex (*HNC*), gyrus rectus (*GR*), cerebellar cortex (*CC*), median raphe nuclei (*MRN*) and medulla oblongata (*MO*). The results are the means ± SEM of 18 observations for the non-smokers and 12 observations for the smokers. Significantly different from non-smokers: * $P < 0.05$; ** $P < 0.01$. □ Non/smokers; ▨ smokers

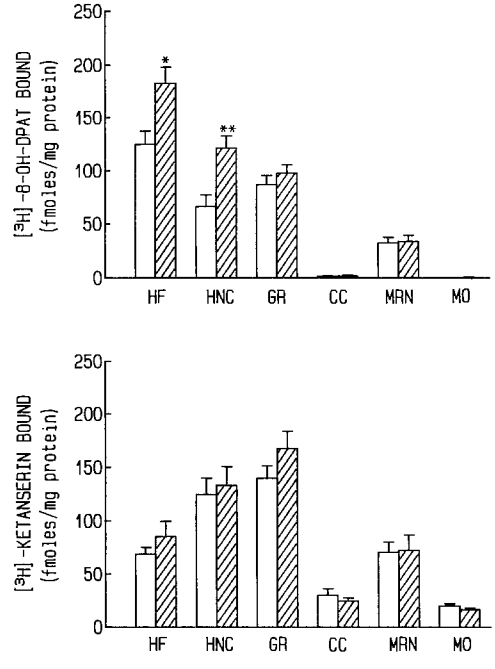

Fig. 2. Radioligand binding to 5–HT receptors in human brain. Binding to membranes prepared from the hippocampal formation (*HF*), the hippocampal neocortex (*HNC*), the gyrus rectus (*GR*), the cerebellar cortex (*CC*), the median raphe nuclei (*MRN*) and the medulla oblongata (*MO*). The results are expressed as means ± SEM of 18 observations for the non-smokers and 12 observations for the smokers. Significantly different compared with non-smokers: * $P < 0.05$: ** $P < 0.01$. □ Non/smokers; ▨ smokers

raphe (Fig. 2). In the cerebellar cortex and medulla oblongata [^{3}H]-8-OH-DPAT binding could not be detected. [3]-ketanserin binding was observed in all brain regions studied, with higher levels in the gyrus rectus, hippocampal neocortex and hippocampal formation than in the median raphe nuclei, cerebellum and medulla oblongata. Smoking was associated with significantly increased [^{3}H]-8-OH-DPAT binding to membranes prepared from the hippocampal formation [F smoking (1,24) = 7.0; $P < 0.05$] and hippocampal neocortex [F smoking (1,24) = 10.3; $P < 0.01$]. Smoking was not associated with any significant changes in the binding of [^{3}H]-ketanserin to 5–HT_2 sites in any of the brain regions studied. However, membranes prepared from the hippocampal formation of male brains bound more [^{3}H]-ketanserin (91 ± ll fmoles/mg protein) [F sex (1,24) = 4.5; $P < 0.05$] than membranes prepared from this region of the brain of females (63 ± 7 fmole/mg protein).

In order to study further the nature of the changes in binding evoked by cigarette smoking, saturation studies on the binding of [^{3}H]-8-OH-DPAT to membranes from the hippocampal neocortex of three smokers were carried out and compared with results obtained with membranes prepared from three age-matched non-smokers. Over the concentration range used, the binding of [^{3}H]-8-OH-DPAT to the hippocampal neocortical membranes was best fitted to a single site model. Analysis of the results of the studies performed with tissue taken from the subjects who had smoked suggested that the increase in radioligand binding to the 5–HT_{1A} receptors evoked by tobacco smoking was associated with an increase in the density of the receptors and also, possibly, their affinity for the radioligand (Table 1). Neither effect, however, reached statistical significance.

[^{3}H]-Ketanserin binding showed no relationship with [^{3}H]-8-OH-DPAT binding in any of the brain regions studied. When the data for non-smokers was considered alone, [^{3}H]-8-OH-DPAT binding to membranes prepared from the median raphe nuclei correlated negatively with the 5–HIAA concentrations in the hippocampal formation ($r = -0.52$, $P < 0.01$). The decrease in the concentration of 5–HIAA seen in the median raphe nuclei with smoking showed a negative correlation with the increase in [^{3}H]-8-OH-DPAT (r = −0.50, $P < 0.01$) binding to hippocampal neocortical membranes which was also observed with smoking (Fig 3.).

The effects of smoking on the [^{3}H]-(-)-nicotine binding to brain membranes are summarised in Fig. 4. The results show that smoking was associated with increased [^{3}H]-(-)-nicotine binding in the hippocampal formation [F smoking (1,24) = 6.1, $P < 0.05$], the hippocampal neocortex [F smoking, (1,24) = 8.9, $P < 0.01$], the gyrus rectus [F smoking (1,24) = 10.1, $P < 0.01$], the cerebellar cortex [F smoking (1,24) = 10.9, $P < 0.01$] and the median raphe nuclei [F smoking (1,24) = 4.7, $P < 0.05$]. No changes were observed in the binding of nicotine to membranes prepared from the medulla oblongata of smokers when compared with non-smokers. The changes in [^{3}H]-(-)-nicotine binding to membranes prepared from the hippocampal neocortex, observed with smoking, correlated

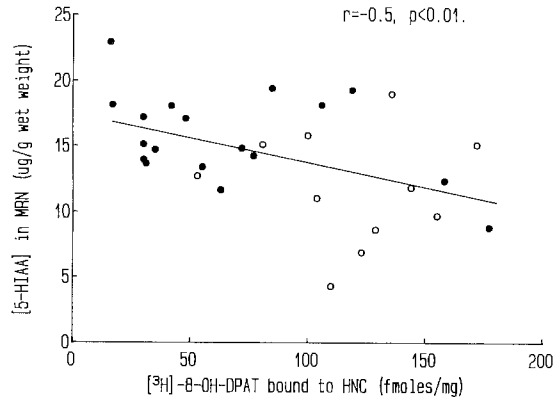

Fig. 3. The relationship between median raphe nuclei 5–HIAA and [^{3}H]-8-OH-DPAT binding to hippocampal neocortex. Data points for the 5–HIAA levels in the median raphe nuclei plotted against the binding of [^{3}H]-8-OH-DPAT to hippocampal neocortex for individual smokers *(open circles)* and non-smokers *(filled circles)*. ● Non/smokers; ○ smokers

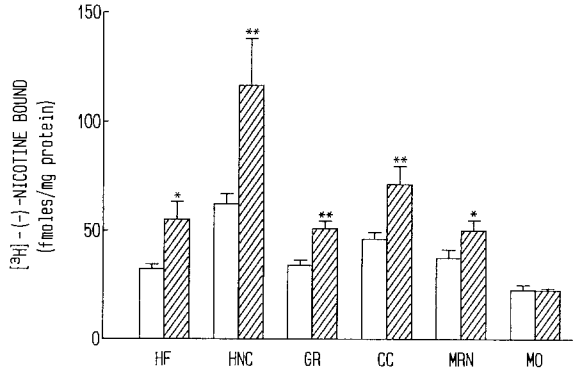

Fig. 4. L-(^{3}H)-(-)-Nicotine binding to membranes prepared from the brains of smokers and non-smokers. The binding of L-(^{3}H)-(-)-nicotine (14 nM) to membranes prepared from the hippocampal formation (*HF*), hippocampal neocortex (*HNC*), gyrus rectus (*GR*), cerebellar cortex (*CC*), median raphe nuclei (*MRN*) and medulla oblongata (*MO*) of smokers and non-smokers. The results are means ± SEM of 18 observations for non-smokers and 12 observations for smokers. Significantly different compared with non-smokers: * $P < 0.05$; ** $P < 0.01$. □ Non-smokers; ▨ smokers

Table 1. Dissociation constants (K_d) and densities (B_{max}) of [^{3}*H*]-*8-OH-DPAT* binding to membranes prepared from human hippocampal neocortex

Smoking status	Age	K_d (nM)	B_{max} (fmole/mg protein)
Non-smoker	62	2.68	173
Non-smoker	79	2.78	212
Non-smoker	74	4.11	231
Mean ± SEM for group	72	3.19 ± 0.46	205 ± 17
Smoker	61	3.54	344
Smoker	79	1.51	234
Smoker	73	1.57	348
Mean ± SEM for group	71	2.20 ± 0.67	308 ± 37

Results are individual values obtained by incubating, in duplicate, hippocampal neocortical membranes with [^{3}H]-8-OH-DPAT at 5 different concentrations (0.3, 0.7, 1.4, 2.6, 5.4 nM)

significantly with the reduction in the levels of 5–HIAA ($r = -0.42$, $P < 0.05$) and also with the increase in [^{3}H]-8-OH-DPAT ($r = +0.45$, $P < 0.05$) binding which occurred in that region as a result of smoking.

Discussion

Pilot studies, using animal tissue to simulate, as closely as possible, the conditions to which the human tissue would be exposed showed prior to analysis, showed that the displaceable binding of [^{3}H]-8-OH-DPAT and [^{3}H]-ketanserin to brain membranes remained stable for at least 96 h postmortem. During this period the 5–HT concentrations tended to decrease to a modest extent (approximately 20%) whereas the concentrations of 5–HIAA tended to increase to approximately the same extent. The highest concentrations of 5–HT and 5–HIAA were found in the median raphe nuclei and medulla oblongata, regions of the brain rich in 5–HT secreting nerve cell bodies. The hippocampal, cortical and cerebellar areas, which receive a serotonergic input from the raphe nuclei but contain no cell bodies, had relatively low concentrations of 5–HT and 5–HIAA. This pattern of distribution is in general agreement with that reported previously by Bucht et al. (1981) and Korpi et al. (1986). In contrast, the hippocampal formation, hippocampal neocortex and gyrus rectus exhibited higher densities of 5–HT_{1A} and 5–HT_2 binding sites than the median raphe nuclei, medulla oblongata and cerebellar cortex, findings which are in broad agreement with those reported by Pazos et al. (1987a and b). Thus these results support the suggestion of Pazos et al. (1987b) that the highest densities of 5–HT binding sites are found in areas of the brain which receive a significant serotonergic innervation rather than the areas which are rich in serotonergic cells and have the highest concentrations of 5–HT and 5–HIAA.

Tobacco smoking was associated with significant reductions in the 5–HIAA levels of the hippocampal formation and neocortex and increases in the radioligand binding to 5–HT_{1A} recognition sites in these two areas of the hippocampus. It is possible that the increase in 5–HT_{1A} receptors could reflect an ability of tobacco smoke to attenuate an age-related loss of the hippocampal neurones on which the 5–HT_{1A} receptors are located. However, the changes are, perhaps, more consistent with tobacco smoking being associated with a reduction in the activity of the serotonergic neurones which innervate the hippocampal region. Smoking was also associated with a significant decrease in the 5–HIAA concentration of the median raphe nuclei, the region of the brain within which the hippocampal serotonergic innervation arises (Moore and Halaris 1975; Bobillier et al. 1976, 1979). In addition, these changes correlated with the increase in binding to the 5–HT_{1A} subtype of serotonergic receptors in the hippocampal neocortex. These data suggest that smoking may be associated with a reduction in the rate of firing of the serotonergic neurones arising in the median raphe nuclei, which innervate the hippocampus.

The physiological function of 5–HT receptors in the brain is not yet clearly understood. However, there is evidence that both 5–HT_{1A} and 5–HT_2 subtypes of the serotonin receptors may be involved in mediating responses to anxiogenic stimuli (Glazer and Traber 1985; Critchley and Handley 1987). Interestingly, buspirone, the prototype of a new group of anxiolytic compounds which selectively act at 5–HT_{1A} binding sites (Glazer and Traber 1983), has also been shown to reduce 5–HT activity of the hippocampus of the rat (Mennini et al. 1986; Higgins et al. 1988; Sharp et al. 1989), probably as a consequence of reduced firing of serotonergic neurones in the raphe nuclei of the rat (Trulson and Arasteh 1986; Higgins et al. 1988). These changes are thought to be involved in its anxiolytic action, which clinically appears comparable to diazepam (Goldberg and Finnerty 1979). The effects of buspirone on the midbrain and hippocampal serotonergic systems are remarkably similar to the changes observed in previous studies with nicotine (Benwell and Balfour 1979, 1982) and in the present study with tobacco smoking. Tobacco smoking is frequently reported to exert a "tranquillising" effect (Gilbert 1979) and there is evidence that nicotine can exert anxiolytic-like activity in some tests (Costall et al. 1989), although the drug is inactive in other tests clearly sensitive to the anxiolytic properties of the benzodiazepines (Morrison 1969; Balfour et al. 1986).

Previous studies in our laboratory have shown that tobacco smoking is associated with an up-regulation of the nicotinic cholinoceptors which are thought to mediate many of the psychopharmacological responses to nicotine (Benwell et al. 1988). In the present study, the increase in nicotine binding sites within the hippocampal neocortex showed significant correlations with both the decrease in 5–HIAA levels and the increase in 5–HT_{1A} binding sites seen in that brain region with smoking. Up-regulation of the nicotinic receptors has also been observed in experimental animals treated chronically with nicotine (Marks et al. 1983, 1985; Marks and Collins 1985: Nordberg et al. 1985). A regionally selective decrease in the serotonergic activity of the hippocampus has also been shown after chronic administration of nicotine to unstressed rats (Benwell and Balfour 1979, 1982).

Thus, it seems reasonable to infer that the nicotine content of tobacco is the agent responsible for the changes seen in this study. However, it seems unlikely that the serotonergic changes are directly related to the changes in the density of the nicotinic receptors, since the relationship is not a particularly close one and studies with experimental animals have shown that chronic nicotine administration reduces hippocampal 5–HT activity when it is given using a treatment regimen which does not cause an increase in the density of nicotinic receptors in the brain (Benwell and Balfour 1985). Unlike the changes in the serotonergic system of the hippocampus, smoking was associated with a more general effect on nicotinic systems since, with the exception of the medulla oblongata, up-regulation of nicotinic receptors was observed in all brain regions studied.

In conclusion, tobacco smoking does appear to be associated with selective changes in the serotonergic system of the hippocampal region of the human brain which

are comparable to those observed previously in rats treated chronically with nicotine. Our data suggests that these changes may occur in response to a reduction in the firing rate of serotonergic neurones arising in the midbrain median raphe. The observations reported in this paper also emphasise the importance of tobacco smoking as one of the factors which might influence brain 5–HT systems when measurements are made at postmortem using human brain tissue.

Acknowledgement. The authors would like to extend their gratitude to the Scottish Home and Health Department for their financial support for this study.

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