to electrical stimulation in these preparations through the stimulation of presynaptic α_2-adrenoceptors.

The present results provide strong evidence that both resorcinol derivatives of octahydrobenzo[f]quinoline are potent dopamine receptor agonists without having presynaptic α_2-adrenoceptor stimulating activity.

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Influence of smoking on serum protein composition and the protein binding of drugs

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The influence of smoking on α_1-acid glycoprotein (α_1-AGP) and serum albumin concentrations and the protein binding of phenytoin and propranolol in healthy volunteers was investigated. α_1-AGP concentrations were found to be statistically different ($P < 0.05$) in the smokers (mean = 84·3 mg dl^{-1}) versus non-smokers (mean = 62·8 mg dl^{-1}). There was a trend for lower serum albumin concentrations and lower fraction unbound of propranolol in the smokers. Smoking did not affect the protein binding of phenytoin.

Studies involving the role of cigarette smoking on drug disposition have focused primarily on the induction of drug-metabolizing enzymes. However, two reports on the influence of smoking on plasma protein binding have been published. In one study (Rose et al 1978), no difference in phenytoin serum protein binding between smoking and non-smoking groups was found. In the other study (McNamara et al 1980), the extent of lidocaine (lignocaine) binding was greater in serum obtained from smokers than in the serum of non-smokers. Although not specifically measured, it was suggested that the cause of this increase in lidocaine binding might be related to elevated concentrations of serum α_1-acid glycoprotein (α_1-AGP) in the smoking population. α_1-AGP is an acute phase reaction protein shown to play an important role in the plasma protein binding of cationic drugs (Piafsky et al 1978) including lidocaine (Piafsky & Knoppert 1979). However, in a multivariable study (Blain et al 1981) in which α_1-AGP was correlated with sex, age, smoking, and the use of contraceptive 'pill', smoking status had no influence on actual serum α_1-AGP concentrations. These observations prompted further investigation. The present communication supports the presence of elevated α_1-AGP levels in the serum of otherwise healthy smokers.

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Methods

Subjects. Serum samples were obtained from 20 healthy, male volunteers, ten smokers and ten non-smokers. Smokers were classified as individuals who smoked more than one pack of cigarettes per day. Non-smokers were individuals who had not smoked for at least two years. The smoking and non-smoking groups were well matched for age (20–45 years old). All subjects were free of any disease or any medication known to cause changes in either serum α_1-AGP concentrations or drug binding to serum proteins. At the time of blood collection, a clinical chemistry profile was obtained using an automatic analyser.

Serum samples. Venous blood was collected in plastic syringes (preliminary studies indicated no influence of these syringes on drug binding) following an overnight fast. The blood was allowed to clot at room temperature for 2 h and then was centrifuged. The serum was collected and stored at −20 °C until used.

Protein binding studies. The serum protein binding of propranolol and phenytoin was determined by equilibrium dialysis using a dialysis membrane (Spectrapor No. 2, Spectrum Medical Industries, Los Angeles, CA) in 1 ml plexiglass cells (Bolab, Inc., Lake Havasu City, AZ). Propranolol and phenytoin were added, in separate volumetrics, to a buffer solution (0·134 M phosphate, pH 7·4) to achieve concentrations of 100 ng ml^{-1} and 15 μg ml^{-1}, respectively. A trace amount of tritiated drug ((±)-4-[^{3}H]propranolol hydrochloride, Amersham, Arlington Heights, IL or 5,5 [phenyl-4-[^{3}H](N)diphenylhydantoin], New England Nuclear, Boston, MA) was also added to the buffer solutions. Radiochemical purity of greater than 98% for both compounds was established by thin layer chromatography. Serum (1 ml) was dialysed in triplicate against

1 ml of one of the buffer solutions for 10 h at 37 °C, previous studies had indicated that 10 h was sufficient time for equilibrium to be established.

Quantitative analysis. Concentrations of drug were quantitated by liquid scintillation counting (Model 3255 Tri-Carb, Packard Instr. Co., Downers Grove, IL) of both buffer and serum aliquots. The average coefficient of variation for individual fraction unbound values from replicate samples was 8·5% for phenytoin and 9·0% for propranolol. Serum albumin concentrations were quantitated by gel electrophoresis (Septratek, Gelman Sciences, Ann Arbor, MI) and α_1-AGP levels were measured by radial immunodiffusion (Mancini et al 1965), using commercially available plates (M-Partigen, Calbiochem-Behring, LaJolla, CA). Statistical analyses were made using the Student's *t*-test.

Table 1. Influence of smoking status on the concentration of serum proteins and on the fraction unbound (f_p) for propranolol and phenytoin.

Subject	Albumin g dl^{-1}	α_1-AGP mg dl^{-1}	f_p (× 10^2) Propranolol	f_p (× 10^2) Phenytoin
Smokers				
1	4·51	85·7	9·13	13·6
2	4·14	87·2	9·05	13·7
3	3·97	90·1	10·8	14·3
4	3·83	59·0	12·7	14·1
5	3·59	105·0	10·1	14·6
6	3·81	88·3	11·8	13·6
7	4·42	90·0	13·7	12·8
8	4·07	78·0	13·6	14·1
9[a]	3·43	160·0	16·3	14·2
10	4·15	75·3	17·6	12·8
Mean	4·05	84·3*	12·1	13·7
±s.d.	±0·29	±12·7	±2·7	±0·6
Non-smokers				
1	4·34	53·8	14·4	14·3
2	4·61	70·0	12·0	14·5
3	4·04	52·8	15·4	15·0
4	4·25	42·9	20·5	14·0
5	4·40	61·5	12·3	14·0
6	4·03	52·8	16·2	13·5
7	4·19	75·7	11·7	12·6
8	4·76	72·7	15·8	11·7
9	4·23	86·7	10·2	14·5
10	4·18	59·0	16·6	13·4
Mean	4·30	62·8*	14·5	13·8
±s.d.	±0·23	±13·3	±3·0	±1·0

[a] Subject No 9 values were not included in the statistical calculations due to abnormal serum chemistry.
* Statistically significant difference ($P < 0·01$).

Results

Clinical chemistry values for all subjects were within normal ranges, except for smoking subject No. 9 who had elevated triglycerides, SGPT (liver transaminase), and lowered bilirubin concentration. The serum protein concentration and binding results for this subject are included in Table 1 but the data are not included in the analysis. Interestingly, this subject had the highest α_1-AGP and the lowest albumin concentration.

The mean serum α_1-AGP concentration was statistically different ($P < 0·01$) in the smokers (84·3 mg dl^{-1}) versus non-smokers (62·8 mg dl^{-1}). There was a non-significant trend for the smoking group to have lower serum albumin concentration (4·05 vs 4·30 g dl^{-1}) and lower fraction unbound of propranolol (0·121 vs 0·145). No difference was observed in the fraction unbound of phenytoin (0·137 for smokers vs 0·138 for non-smokers).

Discussion

The present analysis indicated a trend for the smokers to have lower serum albumin concentrations when compared with non-smokers. However, this difference was not statistically significant [statistical analysis of the data including smoker subject No 9 was significant $P < 0·05$]. A trend for the smoking group to have lower serum albumin concentrations would agree with the results of a study by Dales et al (1974) in which study serum albumin concentrations were correlated with the quantity of cigarettes smoked and a consistent dose-response relationship was shown to exist.

No significant difference was observed in the fraction unbound of phenytoin between the smoking and non-smoking groups. This observation is in agreement with a previously reported study (Rose et al 1978). Since phenytoin is bound primarily to serum albumin (Kinniburgh & Nigel 1981; Porter & Layzer 1975), elevation in serum α_1-AGP should not influence the fraction unbound of phenytoin. The trend toward lower serum albumin concentrations was slight and did not influence the binding of phenytoin.

The mean serum α_1-AGP concentrations were significantly increased in the smoking group (Table 1). Comparison of the individual α_1-AGP concentrations

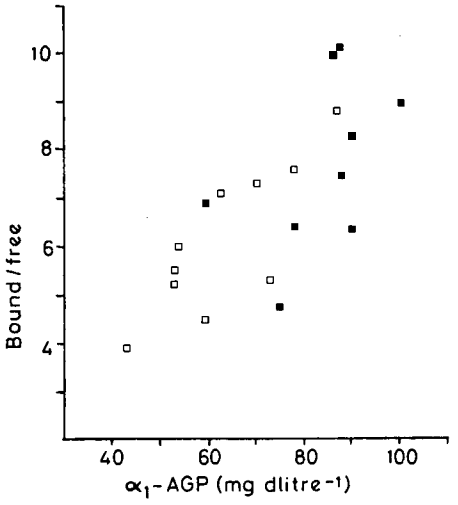

FIG. 1. Influence of α_1-acid glycoprotein (α_1-AGP) on the extent of propranolol binding plotted as bound over free concentration (all data; $r^2 = 0·525$, $P < 0·01$); □ non-smokers; ■ smokers.

showed that there were few overlapping values between the two groups. The underlying mechanism(s) behind the elevation in α_1-AGP in the smoking population is unknown. α_1-AGP has been shown to increase in various disease states (Piafsky et al 1978; Barchowsky et al 1982; Jackson et al 1982), surgery (Fremstad et al 1976), trauma (Edwards et al 1981), and obesity (Benedek et al 1983). Smoking may act in a similar fashion causing an increase in this acute phase reaction protein. Alternatively, smoking may increase α_1-AGP by an enzyme induction mechanism. In adult epileptic patients, long term therapy with phenytoin, phenobarbitone, or carbamazepine was associated with an increased level of serum α_1-AGP (Routledge et al 1981; Tiula & Neuvonen 1982). All of these compounds are known to induce liver enzymes (Greim 1981). Whether these apparent increases in α_1-AGP concentrations in epileptics were the result of enzyme induction and whether a similar induction phenomena is related to the present smoking data is unclear.

The propranolol fraction unbound did not reflect elevated α_1-AGP concentration. A presentation of the data is shown in Fig. 1, where the ratio of bound to free propranolol concentration is plotted against α_1-AGP concentration. This would indicate that the binding of propranolol is strongly related to α_1-AGP serum levels in the non-smokers ($r^2 = 0{\cdot}695$, $P < 0{\cdot}01$). However, α_1-AGP levels in smokers can account for less than 25% ($r^2 = 0{\cdot}227$, $P > 0{\cdot}05$) of the variability in the bound/free ratio.

This lack of influence of α_1-AGP on the binding of propranolol in smokers is rather unexpected, given the usual outcome of elevated α_1-AGP concentrations on the binding of basic drugs in disease states. α_1-AGP was found to be elevated in renal failure, Crohn's disease, arthritis (Piafsky et al 1978), myocardial infarction (Barchowsky et al 1982), cancer (Jackson et al 1982; Abramson et al 1982), and trauma (Edwards et al 1981). In all of these situations the increase in α_1-AGP resulted in a marked increase in the binding of propranolol.

The additional variability in propranolol binding in the smoker might arise from other alterations in serum protein chemistry (i.e. lipoproteins) which bind propranolol (Glasson et al 1980) or the accumulation of endogenous or exogenous substances (i.e. basic compounds in smoke itself) which might compete with propranolol for binding sites. Another possibility is that propranolol may not be binding in the same manner to α_1-AGP in the smokers as it does in the non-smokers.

In the past five years, the importance of α_1-AGP in the binding of cationic drugs has been well established (Piafsky et al 1978; Piafsky 1980). Furthermore, large inter-subject variability in serum α_1-AGP levels has been observed and elevations in this acute phase reaction protein have been attributed to stress, trauma and number of disease states. The clinical significance of elevated α_1-AGP in smokers appears to be small as related to changes in the fraction unbound of propranolol. However, our data suggest that the smoking status of an individual should be added to the growing list of factors which can alter serum α_1-AGP concentrations. Smoking status is a factor worth controlling when designing or evaluating serum portein binding studies.

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