approximately the same in each mixture.

In studies of cell subpopulations isolated from a mouse mammary carcinoma, Heppner *et al.* (*1, 9*) found that specific subpopulations with different growth rates cultured together in monolayer grew at the rate at which one or the other subpopulation grew when cultured alone. We observed a similar effect on the growth of mixed-cell spheroids. Growth curves for untreated spheroids show that 9L spheroids grew faster than R_3 spheroids, yet mixed-cell spheroids grew at the rate of 9L spheroids (Fig. 3). Over the range of spheroid sizes and cell mixtures examined, growth rate was independent of the percentage of 9L and R_3 cells in the initial mixtures (Fig. 3). Because the percentages of 9L and R_3 cells in the initial mixtures were maintained in spheroids with diameters up to 500 μm (Table 1), results suggest that the growth of R_3 cells is increased because of an interaction with 9L cells. The interaction that affects the growth rate of tumor cell subpopulations reported here for spheroids and by others for monolayer culture (*1, 9*) may explain why the fastest growing cell type in a tumor does not become the dominant cell population and why the cellular heterogeneity of the tumor is maintained.

Cell populations of human and animal tumors are heterogeneous with respect to drug sensitivity, growth rate, and other biological characteristics (*10*). Heterogeneity is thought to be a major obstacle to successful cancer therapy. While interactions between tumor cell subpopulations may influence the generation and maintenance of heterogeneity, tumor progression, and response to therapy (*1*), their role in the biological behavior of tumors in situ remains unknown. Use of the spheroid system with the SCE assay to study interactions between cell subpopulations provides a model that in many ways simulates the tumor microenvironment. Moreover, the effects of drug treatment on individual cells can be determined quantitatively. Results obtained with this model may provide a greater understanding of the role of cell-cell interactions in tumor biology.

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Cigarette Craving, Smoking Withdrawal, and Clonidine

Abstract. *Clonidine, an α-2-adrenergic agonist, significantly reduces opiate withdrawal. Fifteen heavy smokers abstained from cigarettes on three separate occasions and received instead clonidine, placebo, or the benzodiazepine alprazolam. Clonidine and alprazolam diminished withdrawal symptoms. The two drugs suppressed anxiety, tension, irritability, and restlessness equally but clonidine had a greater effect than alprazolam on cigarette craving. These observations suggest that noradrenergic activity is a common feature in the pathophysiology of withdrawal and that a special relationship exists between central noradrenergic activity and craving.*

The α-2-noradrenergic agonist clonidine diminishes the opiate withdrawal syndrome in chronically addicted human subjects (*1*). Central noradrenergic function had long been implicated in the action of opiates, but the anatomical locus for that interaction remained unknown. In the 1970's, evidence began to accumulate that a major anatomical connection between the adrenergic and opiate systems existed in the locus coeruleus. This nucleus accounts for nearly half of the noradrenergic neurons and produces the majority of norepinephrine in the mammalian brain. Its noradrenergic cells are densely populated with inhibitory opiate receptors. Enkephalins and opiates as well as α-2-noradrenergic agonists decrease the firing rate of these cells, and abrupt opiate withdrawal results in a marked increase in this firing rate (*2*). Extensive data have now accumulated from both experimental animals (*3*) and man (*4*) confirming Gold's original observation (*1*), and animal data support the assertion that this diminished withdrawal behavior is related to diminished noradrenergic activity (*5*).

We asked whether this concept of noradrenergic involvement in opiate withdrawal could be extended to appetitive behaviors such as smoking. We now report that clonidine alters the acute withdrawal syndrome associated with cigarette smoking and suggest that central adrenergic overactivity is a common feature in the pathophysiology of withdrawal syndromes seen with a variety of addictive substances, including cigarettes, alcohol, and opiates.

Volunteers smoking more than 30 cigarettes per day for at least 1 year, were recruited to participate in a double-blind crossover study of the effects of clonidine on the acute smoking withdrawal syndrome. In addition to a placebo control, a benzodiazepine-like drug, alprazolam, was used in a second experimental condition. Alprazolam has been shown to be equally anxiolytic and slightly less sedative than diazepam (*6*).

All subjects were in good health and were drug-free, except for two female volunteers who used medication for birth control. All subjects were instructed to refrain from smoking for 24 hours on three separate occasions. On each occasion they were told not to smoke after going to bed and to report without smoking at 0830 the next morning. Baseline pulse, blood pressure, and psychological measures were obtained; then one of three treatment regimens was begun. Subjects received clonidine (0.2 mg), alprazolam (1.0 mg), and placebo in one of three randomly assigned sequences. All treatments were given in two divided doses with the second dose given 90 minutes after the first. Pulse and blood pressure, including orthostatic blood pressure, were measured every 90 minutes. At the same time, subjects completed a series of nine visual analog scales. These scales used a 10-cm line to assess tension, anxiety, irritability, craving (thoughts about or wish to smoke), restlessness, impaired concentration, and sadness (or tearfulness). Subjects also completed similar scales for drowsiness and dizziness. At the end of each experimental day, they made a global assessment (on a scale of 1 to 10) of the

Table 1. Mean (± S.E.M.) hour ratings (from an arbitrary analog scale) during hours 2 to 7. Data from hour 1 are excluded because previous blood pressure studies show no drug effects until hour 2 (*16*). There were no significant differences across days at baseline.

Scale	Treatment			Analysis of variance		Friedman	
	Clonidine	Alprazolam	Placebo	*F*	*P*	FTS	*P*
Anxiety	1.46 ± 0.44	1.54 ± 0.46	2.94 ± 0.80	4.53	0.024	4.14	0.12
Irritability	2.20 ± 0.52	2.04 ± 0.77	3.91 ± 0.77	5.34	0.014	7.09	0.03
Craving	3.24 ± 0.67	4.97 ± 0.62	6.03 ± 0.69	9.77	0.001	8.91	0.01
Restlessness	1.60 ± 0.38	1.63 ± 0.37	3.09 ± 0.76	3.69	0.043	4.14	0.12
Concentration	2.17 ± 0.66	1.72 ± 0.52	2.27 ± 0.68	0.05	0.948	0.59	0.74
Sad-tearful	0.83 ± 0.40	0.83 ± 0.30	0.72 ± 0.37	0.04	0.957	3.32	0.19
Tension	1.51 ± 0.35	1.51 ± 0.41	3.12 ± 0.73	5.09	0.016	8.77	0.01
Drowsy	5.06 ± 0.60	4.41 ± 0.70	1.13 ± 0.42	13.10	0.001	13.64	0.001
Dizzy	2.58 ± 0.59	2.04 ± 0.50	1.15 ± 0.35	8.82	0.006	7.95	0.02

degree to which the experimental treatment had helped them to do without cigarettes.

To avoid crossover effects, we asked subjects to resume their normal smoking pattern after each experimental day. A minimum of three normal smoking days separated experimental days. After session 3, subjects were offered a 3-week trial with the experimental treatment of their choice.

Global ratings of the difficulty in not smoking under each of the three experimental conditions were compared by analysis of variance with a randomized block design. The results were further analyzed pairwise by post hoc Studentized range tests. This same procedure was used to compare treatment effects obtained on the nine individual analog scales after averaging the scores during the period from hour 2 to hour 7 after the first doses. A comparison of linear trends on one specific scale, craving, was also accomplished by the same procedure. Because it was not clear that these scale scores represented normally distributed measurements, they were converted to rankings and the more conservative Friedman nonparametric two-way analysis of variance performed.

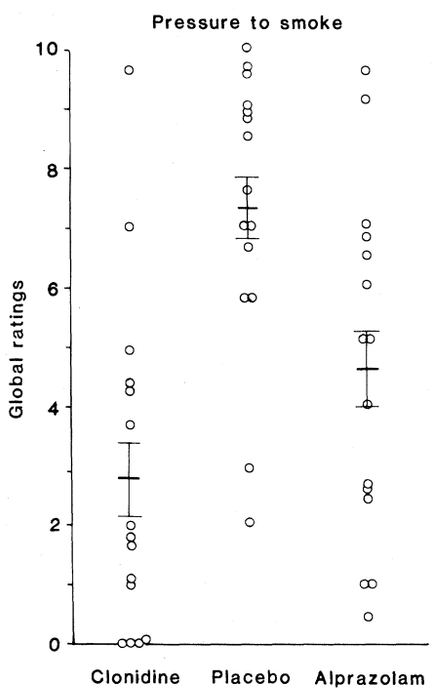

Fig. 1. Individual ratings, mean, and standard errors of the global difficulty in not smoking obtained from subjects after completing all three treatments.

Fifteen subjects, 2 men and 13 women, completed all three experimental days. They averaged 34 years of age, smoked an average of almost two packages per day, and had been smoking for an average of 16 years. Thirteen subjects clearly preferred one of the drug conditions over placebo during the acute phase of smoking withdrawal. Of the two subjects who did not prefer active medication, one showed only modest withdrawal symptoms in the placebo condition and could not distinguish between the drug and placebo conditions, in spite of a history of smoking one and a half packages of cigarettes per day. The other subject who did not prefer drug treatment manifested marked withdrawal symptoms during the placebo period and experienced no relief from either drug. This subject also experienced very little pulse or blood pressure effect from clonidine. Clonidine [$Q(28) = 6.35$, $P < 0.001$] and alprazolam [$Q(28) = 3.76$, $P < 0.05$] significantly reduced the difficulty in not smoking (Fig. 1). Of the 13 subjects who found drug treatment effective, 10 preferred clonidine (binomial calculation, $P = 0.046$).

The analyses of the visual analog scores are presented in Table 1. In spite of the subjects' tendency to prefer clonidine over alprazolam on the global ratings, most individual items did not distinguish between the two drug treatments. For anxiety, irritability, concentration, and tension, both drugs were clearly better than placebo and essentially identical to each other. Even the two most common side effects, drowsiness and dizziness, occurred to the same degree. Only the scale measuring craving (thinking about or wishing to smoke) reflected the strong tendency in the global ratings for subjects to prefer clonidine. Here clonidine was significantly more effective than both placebo [$Q(20) = 6.18$, $P < 0.01$] and alprazolam [$Q(20) = 3.83$, $P < 0.05$]. Alprazolam was not significantly more effective than placebo.

Previous studies of smoking withdrawal syndrome have shown that craving is the most consistently observed withdrawal symptom (*7*) and that it tends to be least in the morning and to increase as the day progresses (*8*). Therefore, we plotted the hourly rating for craving during the first 7 hours of treatment (approximately 0830 to 1530). Craving among

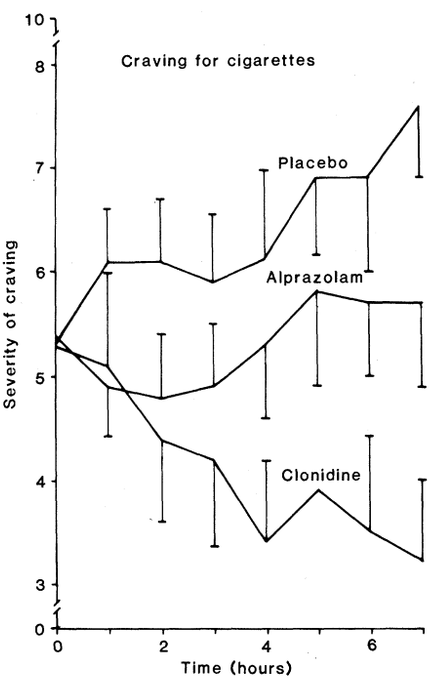

Fig. 2. Mean [± standard error of the mean (S.E.M.)] hourly ratings of craving by treatment. Trend analysis shows mean slope and S.E.M. Clonidine = −0.07 ± 0.13, alprazolam = 0.15 ± 0.11, and placebo = 0.41 ± 0.14. $F(2, 26) = 6.68$, $P < 0.005$ (mean square error, 0.1144). Post hoc test for clonidine versus placebo, $Q(26) = 5.26$, $P < 0.01$.

subjects given placebo increased during the course of the day and, as a result, the contrast between clonidine and placebo increased as the day progressed (Fig. 2).

Clonidine and alprazolam both reduced the acute withdrawal syndrome following sudden abstinence in heavy cigarette smokers. However, subjects preferred clonidine, and clonidine had significantly more effect on cigarette craving than alprazolam. This result does not imply that clonidine is a cure for smoking. Even among those smokers who have been successfully withdrawn from cigarettes, a high percentage will return to cigarettes within the next 6 months (*7, 9*). Nevertheless, any drug that could enable heavy smokers to abstain quickly and relatively asymptomatically is potentially clinically useful. Our clinical experience suggests that clonidine continues to be effective over several weeks; subjects were generally not maintained for a longer period of time. Our experience with alprazolam beyond the brief experimental period was limited and not encouraging.

Several studies have been made of the use of clonidine in the treatment of alcohol withdrawal (*10*). These studies were initially undertaken in an effort to control hypertension associated with alcoholic withdrawal, but clonidine also seems to suppress symptoms of alcohol withdrawal. The efficacy of clonidine in ameliorating cigarette, alcohol, and opiate withdrawal and clonidine's powerful inhibition of noradrenergic activity, together with the evidence from animals of noradrenergic hyperactivity in opiate withdrawal, suggest noradrenergic over-activity as a common characteristic in the pathophysiology of withdrawal syndromes.

It seems reasonable to ask what clinical characteristics these withdrawal syndromes share and to consider how similarities might relate to the efficacy of a drug that decreases noradrenergic activity. Certainly, the delirium tremens and hallucinations of end-stage alcoholic withdrawal differ from the agitation, muscle cramps, and retching of opiate withdrawal and from the tension and irritability of cigarette withdrawal. Before these end-stage characteristics come to dominate the clinical picture, however, these syndromes share certain characteristics. Wikler suggested that the maintenance of opioid-taking behavior depends most on the intense craving for the drug associated with the onset of withdrawal (*11*). Similarly, alcohol withdrawal always begins and usually consists not of delirium tremens but of "bad nerves" and the "urge" for a drink (*11, 12*). In our 15 abstinent subjects, as in other smokers who have been studied (*7*), the most consistent withdrawal symptom is craving, by which we mean a preoccupation with, thoughts about, or an urge for, the habituating substance, not necessarily associated with any physical distress. It is then clear that this craving is a common denominator across these habituations, and we suspect it plays an important role in maintaining the habituation.

Although initial contact with cigarettes, alcohol, or opiates need not be for the purpose of regulating tension or distress, chronic users come to learn that these substances can diminish such dysphoria. They also come to learn that with long-term use the absence of these drugs produces dysphoria. It would be easy to assume that craving for a tension-reducing drug develops in the absence of the drug because the addict experiences a rebound dysphoria and craves or seeks the drug to eliminate that dysphoria. But this is not our observation. Craving is the earliest, the most consistent, and the most severe symptom of cigarette withdrawal. It is also specifically responsive to clonidine. That is, clonidine and alprazolam both reduced anxiety, irritability, restlessness, and tension, and both produced drowsiness; yet clonidine had significantly greater effect on craving. Because clonidine had a greater effect on craving than alprazolam, because it selectively affects noradrenergic activity, and because this noradrenergic effect of clonidine is more powerful than that of the benzodiazepines (*13*), we suggest that there is a special relation between noradrenergic activity and craving.

We hypothesize that the habituated person learns that modest increments in noradrenergic activity predict higher levels of activity (*5*) that are associated with withdrawal dysphoria. Thus, modest increments in noradrenergic function become signals to the addict to seek the substance he has come to expect will diminish his impending distress.

This link between the central adrenergic system and craving is additionally appealing because it explains the observation that stress increases cigarette craving. That is, stress increases central noradrenergic activity (*14*). This heightened noradrenergic activity, even in the absence of any dysphoric feelings, could result in an increasing urge to smoke. It is unnecessary to limit this craving model to habituating substances. Behaviors that reduce tension and noradrenergic activity, such as binge eating (*15*), could be habituating because of a similarly learned craving for the behavior.

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