because of its lack of intrinsic activity.

The affinity of the two analogs for the receptor are less than that of LRF, as is evidenced by the high ($\geqq 10^3$) molar ratios (antagonist/LRF) required for the inhibition of LRF. Because the affinity of des-His^2-LRF for the LRF receptor is the same or higher than that of [Gly^2]LRF, we can propose that either the presence of the imidazole ring or an amino acid in the L configuration in LRF is important but not obligatory for the binding of LRF to its receptor.

The competitive antagonism to LRF by the two analogs, and the lack of LH releasing activity of des-His^2-LRF at a concentration tenfold higher than that required to suppress the response to LRF indicate a dissociation of the binding and secretory processes. This assumption is supported by our observation (*9*) that des-His^2-LRF competes with [^{3}H-Pro^9]LRF for specific binding to anterior pituitary LRF receptors.

Thus the histidyl residue in LRF is somehow required not only for the recognition of LRF by its receptor but for the intrinsic activity of the molecule. Although important, the imidazole ring (or the presence of an amino acid in the L configuration) is not obligatory for LRF intrinsic activity since substitution of glycine for histidine in LRF yields a molecule with almost 50 percent of the LH releasing activity of LRF. However, the peptide linkage in the 2-position seems to be a requisite for the intrinsic activity of the LRF decapeptide, since des-His^2-LRF has little or no LH releasing ability. In the absence of data on the conformation of either LRF or the structural analogs discussed here, we cannot confidently ascertain whether the pharmacological properties of the LRF analogs are a result of alteration of functional groups or are secondary to changes in the conformation of the molecule.

Several proposed hypotheses could explain the observed dissociation of ligand-receptor interactions and subsequent biological responses (*10, 11*). According to the model presented by Changeux and Podleski (*10*) our results could mean that the LRF receptor site and elements mediating the secretory process can exist in two forms in equilibrium: a secretion-triggering state and a resting state, with LRF having preferential affinity for the "secretory" state. The antagonism of des-His^2-LRF would be a consequence of interaction with and stabilization of the LRF receptor in the "resting" inactive configuration. The partial agonist-antagonist [Gly^2]LRF might have affinity for both states, leading at maximum levels to a distribution of the two states determined by its relative binding affinity for the two forms. Of course, other hypotheses which could explain our results based on an induced fit model [see Koshland and Neet (*11*)] are also plausible.

We have previously described a hypothalamic releasing factor analog that probably competes (with a releasing factor) for binding to a biological receptor site: the dipeptide pGlu-His-OMe apparently functions as a competitive inhibitor of the plasma enzyme that inactivates pGlu-His-Pro-NH_2 (TRF) (*12*). Competitive antagonists of the action of other peptide hormones, vasopressin (*13*), angiotensin (*14*), and glucagon (*15*) have been reported. Also, another des-histidine peptide, des-His^1-glucagon, is a competitive antagonist of glucagon (*15*).

These two LRF analogs are the first peptides reported to be competitive antagonists of the biological activity of LRF. The physiological and potential clinical significance of these LRF antagonists is not yet known.

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Effects on Humans of Δ^9-Tetrahydrocannabinol Administered by Smoking

Abstract. *Twelve chronic marijuana users received Δ^9-tetrahydrocannabinol by smoking. The magnitude of their pulse increment was highly correlated with their subjective experiences. Three of the 12 subjects subsequently received Δ^9-tetrahydrocannabinol labeled with carbon-14; the time course of its concentration in plasma was highly correlated with the pulse increment. Subjective symptoms, however, appeared later and dissipated more slowly.*

Numerous studies have been carried out to assess the effects of marijuana (*1*). In many of these studies, natural marijuana or its putative active component, Δ^9-tetrahydrocannabinol (Δ^9-THC), was administered by smoking. Correlations of the concentration of Δ^9THC in plasma with psychological and physiologic effects after administration by smoking were not, however, made.

We report here on a comparison between a 10-mg dose of synthetic Δ^9-THC and placebo marijuana material, both administered to 12 subjects by smoking. The subjective description of effects was qualitatively similar but quantitatively different for the two states. The magnitude of the syndrome as described subjectively by individuals receiving active Δ^9THC correlated very highly with their respective pulse increments.

In order to assess the time course of these variables, we administered to three of the subjects the same dose of

Δ^9THC, a portion of which was in the form of [^{14}C]Δ^9THC. These subjects were then studied for 24 hours. The time course of the pulse rate was highly correlated with the time course of the concentrations of Δ^9THC in plasma. Subjective symptoms, however, appeared later and dissipated more slowly. The setting of the study produced a strong effect on the subjective experience.

The 12 male subjects ranged in age from 21 to 26 years. All had smoked marijuana at least 50 to 75 times; seven had smoked it at least 500 times, and had taken lysergic acid diethylamide (LSD) on one or more occasions. All were free of significant psychiatric or medical illness.

In the initial part of the experiment, each of the 12 subjects smoked a different one of the following on each of 3 days: (i) placebo marijuana material alone, (ii) placebo material injected with 10 mg of synthetic Δ^9THC, or (iii) natural marijuana assayed to contain 10 mg of Δ^9THC (*2*). The order of administration was balanced over the 12 subjects.

All subjects were instructed not to smoke marijuana or to drink alcohol for the 24 hours preceding each experimental day, and not to ingest any other drug for 48 hours preceding that day. Urine samples were analyzed for alkaloids, barbiturates, and amphetamines. These assays were negative in the urine for all subjects.

To assure a consistent pattern of inhalation, only regular cigarette smokers were accepted as subjects. A standard smoking technique was practiced and applied: The subjects inhaled for a period of 2 to 4 seconds, maintained each inhalation for 15 seconds, then exhaled, and waited for 5 seconds. They repeated this procedure until the cigarettes were finished; the time elapsed was 10 minutes. All data are reported in terms of time elapsed from the onset of smoking.

The following observations were made during each session. Radial pulse was measured 30 minutes before smoking; this figure was then subtracted from the pulse rate measured 25 minutes after the onset of smoking to give the pulse increment. Comparative subjective "high" was a single assessment in which subjects rated how "high" they felt during the 90 minutes after the onset of smoking. A scale of 0 to 10 was used in which 0 meant "not high at all" and 10 was the "highest" the subject had ever felt smoking marijuana

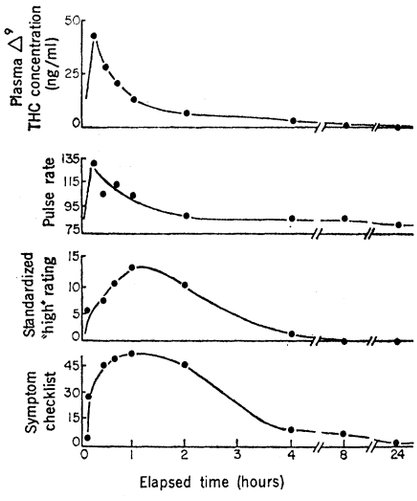

Fig. 1. The time course of plasma Δ^9THC concentration, pulse, and subjective experience in three subjects were studied over the course of 24 hours after they had smoked 10 mg of synthetic Δ^9THC injected into placebo cigarettes. Measurements were taken at zero time, 15 minutes elapsed time (that is, 5 minutes after completion of smoking), 25, 40 minutes, and at 1, 2, 4, 8, and 24 hours.

on any previous occasion. This rating, therefore, reflected the subjects' previous marijuana experiences, as well as the experience of the specific session.

A symptom checklist, a list of 62 subjective symptoms, was given to the subjects after 90 minutes. It was prepared from a questionnaire on subjective drug effects developed by Waskow *et al.* (*3*) for the study of psychoactive drugs. We included items that were found by Waskow *et al.* to differ significantly between 20 mg of orally ingested Δ^9THC and placebo, as well as additional items from the questionnaire, which were relevant to this study (*4*). Each item was graded by the subject from 0 ("not felt at all") to 3 ("felt very much more than usual").

All data were examined by a mixed nested analysis of variance (ANOVA) comparing results for the effects of placebo, Δ^9THC, and marijuana conditions. The Tukey test was then applied for individual mean differences between conditions. Results of both tests were significant, as follows: For the comparative subjective "high" (ANOVA, $F=30.64$; d.f. $=2,22$; $P<.01$), the mean scores were 2.08 for placebo and 5.25 for Δ^9THC ($P<.01$). The mean pulse increment over baseline (ANOVA, $F=21.54$; d.f. $=2,22$; $P<.01$) was 7.3 for placebo and 43.5 for Δ^9THC ($P<.01$). On the symptom checklist (ANOVA, $F=10.03$; d.f. $=2,22$; $P<.01$) scores were 18.0 for placebo and 31.6 for Δ^9THC ($P<.01$).

The subjects' total scores for individual symptoms on the symptom checklist correlated well (Pearson product-moment $r=.94$, $P<.01$) with their subjective "high" rating scores. Similarly, the symptom checklist scores correlated well (Pearson product-moment $r=.70$, $P<.01$) with the magnitude of their respective pulse increments.

Three of the 12 subjects were subsequently studied during 24 hours after they smoked labeled synthetic Δ^9THC. We were thus able to compare the time course of the other variables with that of Δ^9THC concentration in plasma. A total dose of 10 mg of synthetic Δ^9THC, 0.5 mg (10 μc) of which was in the form [^{14}C]Δ^9THC, was injected into two cigarettes prepared from the placebo marijuana material. Blood samples were drawn at the intervals indicated in Fig. 1, and all urine voided was collected. The amount of unchanged Δ^9THC in plasma was determined by extraction at *p*H 7.4 with four volumes of heptane containing 1.5 percent isoamyl alcohol and was assayed for radioactivity by liquid scintillation spectrometry (*5*). Urine was assayed for Δ^9THC and its metabolites by measuring the total radioactivity of the samples.

During the 24 hours after administration of the [^{14}C]Δ^9THC, at the intervals indicated in Fig. 1, baseline and interval radial pulse measurements were made, a symptom checklist was administered, and each subject was asked for a standardized "high" rating. They were now told to grade the "highest" they had felt during all of the smoking sessions as 10 and to grade not feeling "high" at all as 0. If they felt more "high" than they had in the initial part of the study, they were to extrapolate up from this 10-point scale.

Different time courses were found for the variables studied. Both the plasma concentration of Δ^9THC and the pulse increment peaked at 15 minutes, and then rapidly declined (Fig. 1). The subjective experience, however, reached a peak at 1 hour, and declined more slowly. These two different time courses were reflected in product-moment correlations examined over the 24-hour period. Plasma concentration of Δ^9THC showed a significant product-moment correlation with pulse increment ($r=.95$, $P<.01$), but neither of these correlated significantly with the subjective measures. This suggests that the Δ^9THC concentration is more

closely related to changes in pulse than to changes in subjective ratings.

The three subjects absorbed different quantities of Δ^9THC. This variation was apparent in peak Δ^9THC concentrations in the plasma for the three subjects, namely, 67, 37, and 21 ng/ml. An estimate of the portion of Δ^9THC absorbed from the original cigarettes was made from the portion of total tracer present in the 24-hour urine samples. Lemberger *et al.* (*6*) reported that when Δ^9THC was administered intravenously to chronic users, 20 percent of it was excreted in the first day's urine. If the same portion of Δ^9THC and its metabolites administered by smoking is excreted in the 24-hour urine, then our subjects absorbed 41, 20, and 15 percent, respectively, of the quantity of Δ^9THC in the original cigarettes.

Manno *et al.* (*7*), using a mechanical smoking device, assayed the portion of cannabinols in the smoke of marijuana cigarettes, and found that approximately 50 percent of the Δ^9THC in the cigarettes was delivered unchanged in the smoke. This figure is comparable to that of 41 percent absorbed by one of our three subjects, but the other two absorbed less than half that amount. This is an indication of the marked variability of Δ^9THC absorbed when marijuana is administered by smoking, even under standardized conditions.

The subjective experience was responsive to both placebo and change in setting. Although the 12 subjects rated themselves as less "high" on placebo, there was good correlation of symptoms experienced under the influence of placebo and Δ^9THC (rank-order $r = .544$, $P < .01$). The smokers appeared to be conditioned to a particular subjective syndrome triggered by the stimulus of smoking marijuana-like material. This may in part explain the greater sensitivity to marijuana reported by experienced smokers. The 12 most frequently checked symptoms while the subjects were under the influence of Δ^9THC were: mouth drier, feels high, throat drier, hungrier, dreamier, feels more like paying close attention to things, skin tingling, memory seems worse, movements slower, head heavier, sees images when eyes are closed.

When three of the subjects were subsequently studied on the same dose in a more austere setting and subjected to venipucture, the variability in the subjective experience was apparent. Two of the subjects vomited while at the peak of their subjective "high," whereas neither had done so on that dose when it was administered under more congenial circumstances earlier in the study. As one of the two reported on the day of blood drawing, "I just freaked out when I saw that needle." He checked off "very much more than usual" for the symptoms, "have you felt less in control of your body"; "felt less in control of your feelings"; and "had a weird feeling." He had checked "not at all" on the previous occasion.

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Natural Selection for Müllerian Mimicry in Heliconius erato in Costa Rica

Abstract. *The natural color pattern of individuals of the unpalatable and mimetic butterfly* Heliconius erato *was altered to a unique nonmimetic pattern. When returned to natural populations, the nonmimetic individuals remained for shorter periods of time and received more wing damage indicative of predator attacks than did the controls. The results indicate that Müllerian mimicry was functioning to protect the butterflies from predation.*

H. W. Bates and F. Müller, in their theories on protective mimicry, provided the first models of the coevolution of species under the influence of natural selection (*1*). The original theories were deduced from limited empirical evidence; however, a vast body of supportive data has since been accumulated. Most of these data have been natural historical, a posteriori correlative, or based on laboratory experiments (*2*). Direct quantitative evidence supporting the hypothesis that natural selection operates in nature to promote the evolution of mimicry is rare. There exist only one "natural" (*3*) and one manipulative (*4*) experimental field study with living insects demonstrating the selective advantage of Batesian mimicry in nature.

This report describes a set of experiments giving evidence that natural selection is a factor maintaining monomorphism within and similarities between unpalatable species—evidence for the operation of Müllerian mimicry.

Table 1. Comparison of residency time at roosts (= minimum longevity) between altered and unaltered individuals of *H. erato* (Mann-Whitney U test). The entries in the table denote the number of days an individual butterfly was seen returning to the roosts under observation.

	Number of days returning to roost									Mean
				*1968 experiment**						
Altered	52.5	42.5	32	32	27.5	26	10.5			31.7
Unaltered-controls	>71	70	>64	62	57	53	52	22	21	52.4
				1969 experiment†						
Altered	63	47.5	25.5	23.5	22	14	3			28.4
Unaltered-controls	48.5	40	36.5	19	14.5	8	3			24.2

* $U = 13$; $P(U \leq 13) \approx .034$, one-tailed test. † $U = 27.5$; $P(U \leq 27.5) \approx .668$, one-tailed test.