massive serum elevation of endogenous (after implantation) or exogenous human chorionic gonadotropin promotes steroidogenesis and prolongs the life-span of the corpus luteum (*14*) appears to indicate that this type of down regulation may not occur in humans. Although estrogen itself may cause luteolysis (*15*), the twofold increase in estradiol is probably too small and too brief to exert a luteolytic effect (Fig. 1). Moreover, the finding by Gore *et al.* (*15*) that estrogen induces luteolysis in humans only when pharmacologic doses are administered in the period immediately after ovulation tends to exclude estrogen as an intermediary in the observed luteolytic action of LRF agonist. The recent demonstration by Hsueh and Erickson (*16*) that FSH-mediated estrogen and progesterone production by rat and human granulosa cells in culture is markedly inhibited by the addition of LRF as well as by LRF agonist indicates that this hypothalamic peptide may act directly on target cells in the ovary.

Although cellular mechanisms remain to be determined, the present study has demonstrated a luteolytic action of the LRF agonist, and a consequent reduction of corpus luteum steroidogenesis followed by early onset of menses. Since progesterone secreted by the corpus luteum is essential for implantation and the maintenance of early pregnancy (*17*) and since a short luteal phase is causally related to infertility in humans and in rhesus monkeys (*18*), the pattern of administration of LRF agonist used in this study may provide an effective means for the prevention or interception of implantation.

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Erythrosin B Inhibits Dopamine Transport in Rat Caudate Synaptosomes

Abstract. *Erythrosin B is a member of a class of fluorescein dyes that are suggested to elicit hyperkinesis when ingested by susceptible children. We found that erythrosin B inhibits dopamine uptake in rat caudate synaptosomes "uncompetitively" in the 10- to 800-micromolar range. Half maximal inhibition of uptake occurred at 45 micromolar. Uncompetitive inhibition denotes a decrease in efficacy of the dopamine membrane transport mechanism with an increase in affinity of dopamine to the carrier. Erythrosin B also decreased nonsaturable binding of dopamine to the synaptosome membrane. The inhibitory action of erythrosin B on dopamine uptake is consistent with the hypothesis that erythrosin B can act as a central excitatory agent able to induce hyperkinetic behavior.*

Food, drug, and cosmetic (FD&C) dyes have been suggested to play a major role in the etiology of a behavioral disturbance in children called hyperkinesis or minimal brain dysfunction (MBD) (*1*). Controlled behavioral experiments have not revealed a direct or a consistent relationship between FD&C dyes and hyperkinesis (*2*), although there are studies demonstrating that fluorescein-related FD&C dyes are biologically active. Apparently acting by their ability to dissolve in lipid membranes (*3*), FD&C dyes have been shown to affect a variety of biological systems. Eosin B (D&C Red No. 25), eosin Y (D&C Red No. 3), erythrosin B (FD&C Red No. 3), phyloxine B (D&C Red No. 8), and rose bengal inhibit fertilization in the sea urchin *Stronglyocentrotus purpuratus*, and erythrosin B prevents fertilization in members of four other phyla (*4*). Erythrosin B is also active pre- and postsynaptically in frog neuromuscular junction. Presynaptically, erythrosin B increases both the frequency and amplitude of miniature end-plate potentials, possibly by enhancing synaptic membrane and plasma membrane fusion. Postsynaptically, erythrosin B causes an increase in potassium conductance through muscle fiber membranes (*5*). Similarly, erythrosin B and related dyes increase potassium permeability of molluscan neuron membranes (*3*).

The capacity of fluorescein dyes to affect these systems suggests that the proposed relationship between hyperkinesis and food dyes may have a neurochemical basis. We have investigated this possibility by looking at the effects of erythrosin B (FD&C Red No. 3) on dopamine uptake in synaptosomes (*6*) prepared from rat caudate nucleus (*7*). Dopamine uptake is a presynaptic mechanism which terminates the action of dopamine after it is released into the synapse. The dopamine system was selected for study

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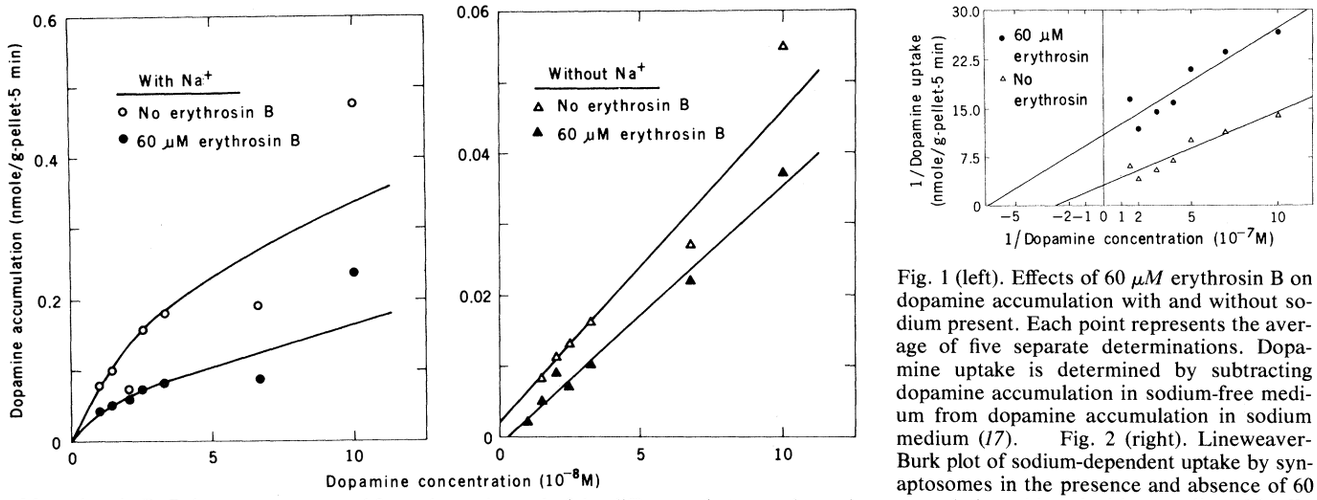

Fig. 1 (left). Effects of 60 μM erythrosin B on dopamine accumulation with and without sodium present. Each point represents the average of five separate determinations. Dopamine uptake is determined by subtracting dopamine accumulation in sodium-free medium from dopamine accumulation in sodium medium (*17*). Fig. 2 (right). Lineweaver-Burk plot of sodium-dependent uptake by synaptosomes in the presence and absence of 60 μM erythrosin B. Points were generated from the reciprocal of the difference between dopamine accumulation with and without sodium. The K_m and V_{max} determinations are calculated from the reciprocal of the x and y intercepts of the lower line, respectively. The K_i is generated from the y intercept of the upper line which is equal to $1/V_{max}(1 + I/K_i)$, where I is equal to the erythrosin B concentration.

because dopaminergic activity has been associated with the motor activity component of the hyperkinetic syndrome (*8*). It was found that erythrosin B significantly decreases dopamine uptake in vitro by inhibiting membrane transport of dopamine.

We used synaptosomes prepared from rat caudate nucleus to measure the uptake of [^{3}H]dopamine in vitro by techniques similar to that described by Wheeler (*9*). The kinetic constants, K_m, representing the carrier's affinity for dopamine, and V_{max}, the maximum velocity for dopamine uptake, were calculated from a Lineweaver-Burk plot (*10*) generated by a National Institutes of Health MLAB computer. The inhibitory constant (K_i) for erythrosin B was generated from a Dixon plot (*11*) as well as the Lineweaver-Burk plot. Straight lines were generated by means of least-squares analysis.

Dopamine uptake by synaptosomes is saturable and sodium-dependent (*12*) with a K_m equal to 33.6 ± 13.8 nM and V_{max} equal to 0.31 ± 0.01 nmole per gram (wet weight) of synaptosome pellet per 5 minutes (Figs. 1 and 2). In the absence of sodium, dopamine accumulation by synaptosomes is linearly related to dopamine concentration in the medium and indicates nonsaturable binding. Erythrosin B decreases both dopamine uptake and nonsaturable binding in synaptosomes. Nonsaturable binding is decreased with 60 μM erythrosin B at all concentrations of dopamine in a parallel manner (Fig. 1). Based on Lineweaver-Burk replotting of the data (Fig. 2), erythrosin B decreases both K_m and V_{max} of dopamine uptake to 13.5 ± 4.4 nM and 0.09 ± 0.01 nmole per gram of pellet per 5 minutes, respectively. Erythrosin B inhibits uptake between concentrations of 10 to 800 μM, and its concentration at half-maximal inhibition (IC_{50}) is 45 μM (Fig. 3A). Independent calculations of K_i for erythrosin B from a Dixon plot (Fig. 3B) and a Lineweaver-Burk plot are 29 and 30 μM, respectively.

The concentration range at which erythrosin B inhibits dopamine uptake, 10 to 800 μM, is comparable to active erythrosin B concentrations at frog neuromuscular junction, 10 to 1000 μM (*5*). Also, the IC_{50} value for inhibiting dopamine uptake, 45 μM, is within the same order of magnitude as the IC_{50} of the same compound in inhibiting fertilization in sea urchin eggs, 14 μM (*4*). The comparable active concentrations of erythrosin B in these systems suggest that erythrosin B is acting consistently on a common feature of these systems.

In the dopamine system, erythrosin B appears to have a dual effect in inhibiting both dopamine uptake and nonsaturable binding to synaptosomes. With sodium present, dopamine uptake is uncompetitively inhibited, as can be determined from the Lineweaver-Burk plot, showing nearly parallel lines for experimental and control conditions (Fig. 2) (*10, 13*). Also known as coupling inhibition, this type of inhibition represents changes in such characteristics as increased enzyme-substrate binding and decreased enzyme effectiveness in yielding products (*13*). This suggests that erythrosin B is blocking dopamine membrane transport while enhancing dopamine binding to the receptor on the carrier. In the absence of sodium, most dopamine uptake is eliminated (*12*). Dopamine accumulation in synaptosomes, observed under sodium-free conditions, results from dopamine binding to other sites on the membrane than those coupled to the carrier (since

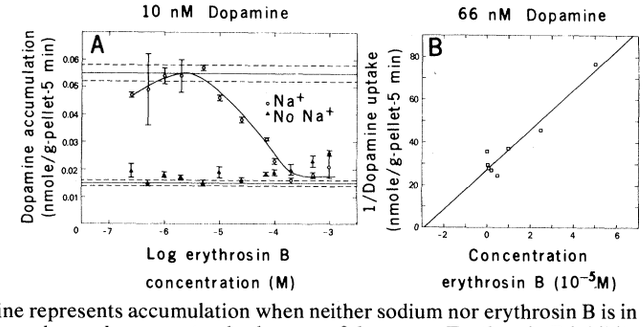

Fig. 3. (A) Dopamine accumulation as a function of erythrosin B concentration, with and without sodium in the medium. Each point represents the average of five separate determinations. The upper solid line represents accumulation with sodium but without erythrosin B present; the lower solid line represents accumulation when neither sodium nor erythrosin B is in the medium. Dashed lines and error bars are standard errors of the mean. Erythrosin B inhibits accumulation with sodium (uptake plus binding) to sodium-free levels (binding only). Discrepancies between the control levels of dopamine binding here and those in Fig. 1 arise from different amounts of protein in the synaptosome suspensions. (B) Dixon plot of (A). Points represent the reciprocal of the difference between dopamine accumulation when sodium is or is not present. The value of the K_i was generated from the negative of the x intercept which is shown to equal 30 μM.

this binding was not saturable). Erythrosin B could be blocking this nonsaturable binding by altering membrane fluidity and thereby decreasing the stability of the dopamine-synaptosome complex (*14*). Contrary to this speculation, increasing dopamine accumulation occurs in the absence of sodium at high erythrosin B concentrations (Fig. 3A). But this apparent increase in nonsaturable binding is likely to be artifactual since erythrosin B is present as a disodium salt, and at increasing erythrosin B concentrations sodium levels are also increased, thereby stimulating sodium-dependent dopamine uptake.

We can conclude that erythrosin B is acting as a significant dopamine uptake inhibitor when present with brain tissue in vitro. Compared with amphetamine, another known inhibitor of dopamine uptake in rat striatum (*15*), erythrosin B is about one-hundredth as potent in inhibiting dopamine uptake. It cannot be assumed that erythrosin B can inhibit dopamine uptake in vivo, since it is not known whether erythrosin B penetrates the blood-brain barrier or is present in brains of animals after peripheral administration or oral ingestion. Nor can it be assumed that erythrosin B is a specific inhibitor of dopamine uptake since a wide scope of effects by erythrosin B has been demonstrated by previous studies (*3–5*). However, it has been shown (*16*) that sodium-dependent glutamate uptake in synaptosomes prepared from rat cortex is not inhibited by erythrosin B. In fact, it has been found that low concentrations of erythrosin B (50 n*M*) actually increase sodium-dependent glutamate uptake. These recent observations suggest that erythrosin B is acting specifically on the dopaminergic system in inhibiting uptake. This is consistent with increased dopaminergic activity in vivo, which has been suggested to be involved with the hyperkinetic syndrome.

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18. We thank H. Levitan for critically reading this manuscript and providing invaluable advice during the course of experimentation.
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Adaptive Female-Mimicking Behavior in a Scorpionfly

Abstract. *This study provides a clear example of female-mimicking behavior by males in insects and evaluates quantitatively the adaptive significance of this behavior, which is poorly understood in many other organisms. Males of* Hylobittacus apicalis *provide females with a prey arthropod during copulation. Some males mimic female behavior when interacting with males that have captured nuptial prey, resulting in males stealing prey which they will use for copulation. Males that pirate prey copulate more frequently and probably incur fewer predation-related risks.*

Natural selection theory predicts that intraspecific deception will be common between potential and actual mates, parents and offspring, relatives in general, and indeed between all socially interacting individuals of all animal species. According to theory, selection will favor individuals that can deceive other individuals because the deceiver may gain time or resources, both of which can be used for reproductive activities, or the deceived may be used to enhance directly the reproductive success of the deceiver (*1*). Theory predicts that small lies will be prevalent over gross lies because selection would favor individuals that can detect deception (*2*). Transvestism is "the practice of adopting the dress, the manner, and frequently the sexual role of the opposite sex" (*3*). It may be a common form of deception in animals. Apparent transvestism has been reported in humans (*4*) and a few other primates (*5*), hyenas (*6*), mountain sheep (*7*), birds (*8*), salamanders (*9*), and several fishes (*10, 11*). Speculative adaptive explanations for mimicry of the opposite sex have been provided for most of these cases. The suggested reproductive advantage incurred by transvestite males in fish species with external fertilization is the least equivocal. Males without territories adopt or retain female coloration and are observed to "sneak" into other male territories and release sperm while a female is spawning. Presumably the transvestite steals fertilizations from the territorial male (*11*); however, sneaking may be merely a complex courtship tactic in some fish (*12*).

Transvestism has not been reported in insects (*13*). I report here female-mimicking behavior of adult males of the scorpionfly *Hylobittacus apicalis* (Mecoptera: Bittacidae) (*14*) that clearly enhances the copulatory success and probably the survival of these males. *Hylobittacus apicalis* preys on arthropods. Males exhibit nuptial feeding; that is, a prey arthropod is fed to the female during courtship and copulation (Fig. 1). In hunting for prey, males expend time and are exposed to web-building spider predators. Transvestites rob males of their prey, reducing their own hunting time and risks. The reduction in hunting time allows transvestites to copulate more frequently (*15*).

The sequence of sexual behavior in *H. apicalis* begins when a male acquires a prey arthropod—through his own predatory activites or by stealing—and begins feeding on it. After feeding briefly he either discards the prey and obtains anoth-

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