showed no more inhibition of 5′-nucleotidase than the non-psychoactive (+)-Δ^8-THC. The very lipophilic synthetic cannabinoid dimethylheptyl Δ^9-THC was not significantly more inhibitory than the other cannabinoids.

It has been observed that the partition coefficient (x) of a compound between an organic phase and water can be estimated from the characteristic volume V_x by the relationship

$$\log_{10}x = kV_x - E_B \quad (1)$$

where k is a constant and E_B is an interaction term to account for specific interactions (usually by polar groups) which increase the solubility of the compound in one or both liquid phases [11]. The constant k is close to 36,000 moles M^{-3} at 298° K for most organic liquids and is largely determined by the properties of water. The relationship (1) has been used to estimate partition coefficients of steroids between ether and water [10]. From Equation 1 an expression has been derived [12] relating the toxic dose C_t of drug to its characteristic volume V_x:

$$C_t = A + [B \times 10^{-36{,}000\ V_x} + E_B] \quad (2)$$

In Equation 2 A and B are constants for the system and the ratio A/B is equal to the ratio of volume of non-aqueous phase/volume of aqueous phase.

In Fig. 1 the $-\log K_i$ values for the inhibition of 5′-nucleotidase have been plotted as a function of the characteristic volume V_x. The plot indicates that the inhibition follows that expected for physical toxicity with the curve having the form of Equation 2. Only dodecanol and trans-retinol show much deviation from the curve, in both cases giving less inhibition than expected for physically toxic compounds. When Equation 2 is applied to the curve of Fig. 1, the value of the constant A is 1.78×10^{-5} moles/l and the value of the constant B is 1.78 moles/l, this being the concentration of the drugs required in the biophase for 50% inhibition. The value of $A/B = 1 \times 10^{-5}$, which from Equation 2 is the ratio of the volume of the biophase to the volume of cell suspension, so that 10 μl of biophase is present in 1 liter of cell suspension. This is in accord with the calculated volume of plasma membrane lipid in lymphocyte suspensions [8].

The lack of specificity in the inhibition of mouse lymphocyte 5′-nucleotidase by cannabinoids does not exclude the possibility that neuronal 5′-nucleotidase may be susceptible to specific effects. Similar physicochemical correlations may be applied to the inhibition of other membrane-bound enzymes such as brain ATPases or 5′-nucleotidase by cannabinoids to determine whether such effects are related to the psychoactivity of these compounds.

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Guelph-Waterloo Centre for Graduate Work in Chemistry
University of Guelph
Guelph, Ontario N1G 2W1
Canada

MARGOT C. SADLER
FRANCES J. SHAROM
ALAN MELLORS*

* Author to whom all correspondence should be addressed.

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Quipazine and induction of adrenal enzymes

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Two enzymes of the rat adrenal medulla, tyrosine hydroxylase (TH, EC 1.14.16.2), the rate-limiting enzyme in the biosynthesis of catecholamines, and ornithine decarboxylase (ODC, EC 4.1.1.17), the first enzyme in the polyamine biosynthetic pathway, are induced following repeated administration of dopaminergic agents [1–5]. These drugs act supraspinally. There is also evidence that serotonin-containing fibers originating in the medial raphe nucleus play a role in this process [4, 6].

Another type of drug that acts supraspinally in the rat to cause an increase in adrenal TH activity is the piperazine-substituted quinoline derivative quipazine [7]. This substance has a variety of actions relating it to central dopamine functions [8–10]. Nevertheless, the stimulatory action of quipazine on adrenal TH activity does not immediately fit with its other effects that are related to serotonin functions: binding to postsynaptic sites that mediate inhibitory action of this amine [11, 12]; facilitation of serotonin release [13]; inhibition of its uptake [14]; and inhibition of type A monoamine oxidase [15], the form of this enzyme that acts physiologically on serotonin.

To resolve this apparent contradiction in the action of quipazine, we have tested this drug in animals previously given a serotonin-receptor blocking agent in order to leave the dopamine-sensitive sites available. The blocker we have used is methiothepin, a substance shown earlier [2] to cause

increases in adrenal TH activity. Although pharmacological experiments suggest that methiothepin blocks both serotonin and dopamine receptors, it is difficult to attribute the effect on adrenal TH to blockade of catecholamine receptors because haloperidol, which blocks dopamine and also noradrenaline receptors to some extent, has no effect on adrenal TH activity [2, 16]. On the other hand, the increase in enzyme activity following methiothepin is consistent with other evidence [2] for the role of a serotonergic system with a net inhibitory action in the regulation of adrenal TH.

The parallel (but much more rapid) induction of adrenal ODC allowed us to examine the effects of the test drugs on two more systems: adrenomedullary ODC which, like TH, is under neural control; and ODC of the cortex, which responds to pituitary ACTH [17, 18].

Methods and materials

Tyrosine hydroxylase experiment. The interaction of quipazine with methiothepin in regard to TH induction was carried out in two experiments with twenty-five and twenty-four male Sprague–Dawley rats respectively. The animals weighed (mean ± S.E.) 200 ± 20 g. Groups of five to seven animals were injected intraperitoneally with methiothepin, 15 mg/kg, given at zero time; quipazine, 10 mg/kg, given subcutaneously twice at 0 and 6 hr; or both drugs. This regimen was repeated on days 2 and 3. The animals were killed 17 hr after the last injection of quipazine by intraperitoneal administration of sodium methohexital (65 mg/kg) in the first experiment or sodium pentobarbital (60 mg/kg) in the second experiment. The endogenous TH activity was similar in each experiment, as were the other results, which were then pooled for purposes of statistical analysis.

The adrenals were removed, freed from capsular tissue, weighed and homogenized in 0.5 ml of ice-cold saline. Adrenal TH activity was assayed according to the method of Nagatsu *et al.* [19], as modified by Gauthier *et al.* [3].

Adrenal ODC experiments. The interaction of quipazine and methiothepin in regard to ODC induction was carried out with twenty male rats weighing 205 ± 5 g. Groups of five animals were injected at 15 min before zero time with methiothepin, 15 mg/kg, given intraperitoneally; quipazine, 10 mg/kg, given subcutaneously at zero time; or both drugs. The rats were killed by decapitation at 4 hr. The adrenals were quickly removed, freed from capsular tissues, as before, and weighed. Removal of the capsule results, as is well known, in loss of the zona glomerulosa. The separation of the adrenal medulla from the cortex was done at 4° with fine scissors, under a magnifying lamp. The portion of the tissue corresponding to two medullae or two cortices was pooled and homogenized in sodium-potassium phosphate buffer, 0.05 M, pH 6.8.

ODC activity was determined by a method that combines elements of the assays described by Russell and Snyder [20] and Jänne and Williams-Ashman [21], with some minor modifications [22].

Statistical analysis. Values in all tables are expressed as mean ± S.E. Comparison of paired means is based upon Student's *t*-test [23].

Results

TH activity. The data in Table I show that methiothepin and quipazine, respectively, caused small increases in adrenal TH activity: the increase with methiothepin was statistically significant ($P < 0.05$). The result with quipazine, which in this case was not significant, is smaller than that obtained earlier by Gagner *et al.* [7]. In rats receiving the two drugs, there was a highly significant increase in the adrenal TH activity ($P < 0.001$), such that the two drugs are seen to potentiate one another. The increase by methiothepin alone and the larger increase when given together with quipazine are consistent with the previous finding that methiothepin invokes a dose-dependent increase of adrenal TH activity [2].

Adrenomedullary ODC activity. The results in Table 2 show that a single injection of quipazine produced a 4-fold increase in adrenomedullary ODC activity at 4 hr ($P < 0.05$). Methiothepin caused a 3-fold increase in this activity. Combination of the two drugs resulted in a 10-fold increase of ODC activity over controls ($P < 0.01$). This clearly indicates that methiothepin potentiates the action of quipazine.

Adrenocortical activity. When ODC was measured in the adrenal cortex 4 hr after the last injection of quipazine, there was a 5.5-fold increase of activity ($P < 0.001$), as can be seen in Table 2. The administration of methiothepin did not have an effect on cortical ODC ($P < 0.05$) but, when this drug was used in combination with quipazine, it totally prevented the increase with quipazine alone.

Discussion

It has been shown previously that a serotonergic system, originating in the medial raphe nucleus, exerts a net inhibitory action over the activity of adrenal TH [2]. Experimental procedures that interfere with the function of that system result in increases of the enzymic activity. In this context, if quipazine were acting as a serotonin agonist, one would have expected it to neutralize the mild inductive effect of methiothepin functioning in this instance as a serotonergic antagonist (see beginning of paper). On the contrary, as the results in Table 1 show, quipazine potentiated the action of methiothepin. This is consistent with claims that quipazine can exert dopaminergic actions [8–10].

In regard to the experiment in which adrenomedullary ODC was measured, there was again potentiation seen with the combination of methiothepin and quipazine. This is analogous to the previously reported potentiation of the action of apomorphine on induction of adrenomedullary ODC by prior treatment of the animals with either *p*-chlorophenylalanine, an inhibitor of the synthesis of serotonin in the nervous system, or with 5,7-dihydroxy-

Table 1. Effects of quipazine and methiothepin on tyrosine hydroxylase activity of the adrenal gland

Drug treatment	Adrenal TH activity [nmoles DOPA · hr^{-1} · (pair of glands)$^{-1}$]	
Saline	44.6 ± 1.8*	(12)
Quipazine	50.0 ± 2.5	(12)
Methiothepin	53.2 ± 2.7†	(11)
Both	67.6 ± 4.0‡,§	(14)

* Mean ± S.E. (number of animals in parentheses).
† $P < 0.05$ vs saline.
‡ $P < 0.001$ vs saline.
§ $P < 0.05$ vs methiothepin.

Table 2. Effect of methiothepin on adrenal ODC induction by quipazine

Drug treatment	Adrenal ODC activity [pmoles CO_2·(mg protein)$^{-1}$·(45 min)$^{-1}$] A. Medulla	B. Cortex
Control	33 ± 5* (5)	14 ± 3 (4)
Quipazine	139 ± 38† (5)	80 ± 1‡ (5)
Methiothepin	99 ± 21§ (5)	18 ± 9§ (4)
Both	326 ± 73‖ (5)	19 ± 6¶ (5)

* Mean ± S.E. (number of animals in parentheses).
† $P < 0.05$ vs controls.
‡ $P < 0.001$ vs controls.
§ Not significant vs controls.
‖ $P < 0.05$ vs quipazine-treated rats.
¶ $P < 0.001$ vs quipazine-treated rats.

tryptamine, a neurotoxin acting (by intracerebroventricular injection) on cerebral serotonin-containing neurons. If quipazine were acting as a serotonin agonist, one would expect its action to be impaired by the administration of methiothepin. Hence, the results in Table 2 suggest once again that quipazine is producing an increase in the ODC activity of the adrenal medulla through a dopaminergic action.

The regulation of adrenocortical ODC is somewhat different from the other two adrenal enzymes measured in this work. Although it is induced by dopamine agonists, an intact serotonergic system originating in the medial raphe nucleus seems *necessary* for that effect [24]. The results in Table 2 for adrenocortical ODC are consistent with such a mechanism. Thus, the inductive action of quipazine on adrenocortical ODC is consonant with a dopaminergic action of that drug, as in the previous cases. Further, the blocking of that induction by methiothepin is an effect that is achieved by a serotonin antagonist acting on facilitatory neurons to prevent their participation in the neural induction of the enzyme.

To summarize, the effect of quipazine on the activity of three adrenal enzymes that are induced by dopaminergic agents, but which respond characteristically and differentially to modification of serotonergic activity, has been studied. Rats received quipazine alone or in combination with methiothepin, a serotonin antagonist. The results demonstrate that the action of quipazine in the induction of the three enzymes is consistent with a dopaminergic effect.

* Address correspondence and reprint requests to: T. L. Sourkes, Ph.D., McGill University, Department of Psychiatry, 1033 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1.

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Departments of Biochemistry and Psychiatry
McGill University
Montreal, Quebec, Canada

MARC EKKER
KRYSTYNA MISSALA
THEODORE L. SOURKES*

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