INDOLEAMINE ANTAGONISTS: RELATIVE POTENCIES AS INHIBITORS OF TRYPTAMINE- AND 5-HYDROXYTRYPTOPHAN-EVOKED RESPONSES

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Four indoleamine antagonists were evaluated for relative potencies as inhibitors of tryptamine-induced forepaw clonus and 5-hydroxytryptophan-evoked head twitches. Methergoline was approximately three times more potent against the forepaw clonus than the head twitch response, whereas methysergide exhibited nearly equal activity in both tests. Cyproheptadine and cinanserin showed a profile opposite to methergoline and a greater degree of selectivity, being 25 to 40 times more potent as inhibitors of the 5-hydroxytryptophan- than of the tryptamine-induced response. These findings clearly demonstrate that the rank order of potency of indoleamine antagonists varies greatly depending upon the test procedure employed.

Two pharmacological responses frequently employed for identification and characterization of compounds which block the actions of indoleamines on the central nervous system (CNS) are the head twitch response provoked by administration of 5-hydroxytryptophan (5-HTP) and the clonus of the forepaws induced by injection of tryptamine. Even for well-known indoleamine antagonists such as cyproheptadine (Stone, Wenger, Ludden, Stavorski & Ross, 1961), cinanserin (Rubin, Piala, Burke & Craver, 1964), methergoline (Ferrini & Glasser, 1965) and methysergide (Doepfner & Cerletti, 1958), information obtained in the same species regarding their relative potencies in antagonizing the 5-HTP-induced and tryptamine-provoked responses is not readily available. Although it might seem likely *a priori* that compounds highly active as inhibitors of one response would also be similarly active against the other, the results of the present study indicate that certain indoleamine antagonists actually exhibit large differences in their relative activities as inhibitors of the tryptamine- and the 5-HTP-evoked responses.

Methods Female Sprague-Dawley rats (170-210 g) were used in both tests. Treatments and doses were randomized and observations were made on a blind basis. Five to twenty rats were used at each of four or more dose levels of each antagonist tested.

5-hydroxytryptophan-elicited head twitch The head-twitch response was assessed on a quantal basis as previously described in mice by Corne, Pickering & Warner (1963). The antagonists were administered 30 min prior to 5-HTP (270 mg/kg) and the number of animals exhibiting at least one head twitch was determined 1 h later. In animals not receiving an antagonist, this dose of 5-HTP caused head twitches in 100% of the rats.

Tryptamine-induced forepaw clonus Using 5 s or more of uninterrupted clonus as the end point, the antagonists were evaluated for their ability to inhibit tryptamine-induced clonus of the forepaws (Tedeschi, Tedeschi & Fellows, 1959). The test compounds were given 1 h prior to tryptamine (40 mg/kg). Clonus of the forepaws was observed in 100% of the animals not receiving the antagonist drugs.

In both test procedures, the dose of antagonist required to prevent the response in 50% of the rats (ED_{50}) was estimated by regression analysis.

With the exception of tryptamine, which was administered *via* the tail vein, all drugs were injected intraperitoneally. All doses are expressed in terms of the base. Cinanserin hydrochloride, methysergide maleate and methergoline were generous gifts from E.R. Squibb & Sons (New Brunswick, N.J.), Sandoz Pharmaceuticals (Hanover, N.J.) and Farmitalia (Milan, Italy) respectively. Cyproheptadine hydrochloride (Periactin) is a product of Merck & Co. (West Point, Pa.). Tryptamine hydrochloride and DL-5-hydroxytryptophan were purchased from Calbiochem (Los Angeles, Calif.) and Aldrich Chemical Co. (Milwaukee, Wis.), respectively.

Results Cyproheptadine and cinanserin were considerably more effective in antagonizing 5-HTP-induced head twitches than as antagonists of tryptamine-induced clonus (Table 1). Methergoline, on the other hand, was more potent as an antagonist of tryptamine than of 5-HTP, whereas methysergide was nearly equally effective in both tests. With regard to antagonizing 5-HTP-induced

head twitches, the order of potency (cyproheptadine > methergoline > cinanserin > methysergide) was quite different from the order of activity of these compounds as inhibitors of tryptamine-induced clonus, which was, methergoline > methysergide > cyproheptadine > cinanserin.

Discussion Previous studies have established that 5-HTP-provoked head twitches and tryptamine-induced clonus are, respectively, produced by an action on the C.N.S. of 5-hydroxytryptamine (5-HT; serotonin) and tryptamine (Tedeschi *et al.*, 1959; Corne *et al.*, 1963; Mawson & Whittington, 1970). Therefore, reference is made subsequently to the relative central anti-5-HT and anti-tryptamine activities of the various antagonists in the two test procedures.

Methergoline stands out as being the only antagonist with a high degree of potency in blocking both the tryptamine- and 5-HT-evoked responses. Methysergide, like methergoline, is structurally related to lysergic acid diethylamide and demonstrated nearly equal potency in both the antitryptamine and anti-5-HT tests. Cyproheptadine and cinanserin, which are neither structurally related to each other nor to methysergide or methergoline, were notable because of their selectivity, being about 25-40 times more active against the 5-HT-induced than the tryptamine-induced response.

To explain the differences observed among the various antagonists, pharmacodynamic factors such as distribution or duration of effect no doubt play some role. However, it seems unlikely that variables of this type will adequately account, for example, for the 140-fold (42/0.3, Table 1) separation in the relative potencies of cyproheptadine and methergoline as antagonists of the tryptamine- and 5-HT-induced responses. The existence of more than one type of receptor for tryptamine-like substances has been proposed as an explanation for the variation in the susceptibility of peripheral tissues to indoleamine antagonists (Gaddum & Picarelli, 1957; Gyermek, 1965; Vogt, 1968). A similar situation in the CNS could account for the results of the present study.

If future studies reveal the same order of potency and selectivity for the antagonists used here against other responses evoked by tryptamine or 5-HT, the antagonists would provide the basis for a convenient pharmacological test for distinguishing between physiological or pharmacological effects in the CNS mediated by endogenous tryptamine and 5-HT. In any event, it is clear from these results that the potency of well-known indoleamine antagonists depends greatly upon the test procedure employed. This fact should be considered when interpreting data from studies using these compounds as aids in elucidating the functional roles of brain 5-HT, tryptamine and other endogenous indoleamines.

Table 1 Comparative potencies of indoleamine antagonists.

Response	ED_{50} (mg/kg) (95% confidence limits)			
	Cyproheptadine	*Methergoline*	*Cinanserin*	*Methysergide*
5-HTP-induced head twitch	0.12 (0.03-0.44)	0.47 (0.12-2.10)	1.5 (1.2-1.8)	2.5*
Tryptamine-induced forepaw clonus	5.0*	0.14 (0.07-0.29)	>40.0	2.3 (1.9-2.6)
Ratio: Tryptamine/5-HTP	42	0.3	>27	0.9

5-HTP = 5-hydroxytryptophan.
* Dose-response curve was not suitable for estimation of 95% confidence interval, since greater than 60% antagonism was not observed even at doses as high as four times the ED_{50} value.

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