Anticholinergic Activities of Imipramine and Methylphenidate

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Abstract □ Anticholinergic activity was measured in anesthetized cats by the antagonism of acetylcholine and McN-A-343. Imipramine and methylphenidate blocked the sialagogic and vasopressor responses to McN-A-343 in doses about one-tenth of that required to block the vasodepressor responses of McN-A-343 or acetylcholine. Atropine blocked the four cholinergic responses at the same dose level.

Keyphrases □ Anticholinergic agents—activity □ Imipramine—anticholinergic activity □ Methylphenidate—anticholinergic activity

Osborne and Sigg (1) reported that imipramine blocked the fall in blood pressure from peripheral vagal stimulation in doses that did not affect the vasodepressor effect of an intravenous injection of acetylcholine. Methylphenidate in comparatively higher doses had similar effects. The authors felt that the blockade of vagal stimulation was the result of conduction anesthesia and not from an atropine-like activity. Peripheral cholinolytic activities of imipramine-like antidepressants, however, have been repetitively shown, both clinically and in laboratory animals, *e.g.*, Kline (2), Rathburn and Slater (3). Some investigators have also attempted to correlate central cholinolytic activity in animals with antidepressant efficacy in man (4, 5). In the present study, the authors compare the anticholinergic activities of imipramine and related compounds in several distinct muscarinic responses from the same animal: vasodepression from acetylcholine, vasodepressor, pressor, and sialagogic responses from McN-A-343. The latter compound has been shown to cause a rise in blood pressure through an atropine-sensitive stimulation of the sympathetic ganglia and adrenal medulla (6, 7). It also causes saliva secretion from the submandibular gland by a mechanism independent of its ganglionic effects (8). The results of this study show that cholinergic responses have differential sensitivities to antagonists which should be taken into account when attempts are made to predict related effects in man or to infer mechanism of action.

METHODS

Cats of either sex weighing 2–4 kg. were anesthetized with sodium pentobarbital (35 mg./kg., i.p.). After bilateral vagotomy, a femoral artery and a femoral vein were cannulated for blood pressure recording and intravenous injections, respectively. The submandibular (Wharton's) duct was cannulated with a fine polyethylene tubing. The saliva was collected in a small cup, suspended from a force-displacement transducer.[1] The increase in weight in the cup and arterial blood pressure were recorded on a polygraph.[1]

[1] Force-displacement transducer, Grass FT.03, and polygraph, Grass model 79.

Drugs were dissolved in normal saline and administered intravenously in the following doses and sequence: acetylcholine iodide, 0.5 mcg./kg.; McN-A-343, 0.1 mg./kg. After control responses were recorded, the test drugs were administered and the sequence repeated. Three doses of each test drug were given in cumulative doses progressed at a 0.5-log interval. Five animals were used for each test drug and the result was analyzed by Dunnett's *t* test.

RESULTS

The effects of atropine, imipramine, methylphenidate, and cocaine on the responses to muscarinic stimulation are summarized in Fig. 1.

Atropine, in the very low dose of about 3 mcg./kg., reduced all four responses of acetylcholine and McN-A-343 to between 50 and 70% of that of control. At higher doses of atropine, all four responses were nearly completely suppressed. The log dose-response curves of atropine on four cholinergic responses are nearly identical.

Imipramine and methylphendiate appeared to have a more selective effect on some muscarinic responses. At the low doses of either compound, the vasopressor and sialagogic responses of McN-A-343 were reduced to 30–60% of that of control. Similar degrees of antagonism in the vasodepressor responses of acetylcholine and McN-A-343 were attained only with about 10-fold the lower dose. A slight enhancement in the depressor responses was observed in a dose of impiramine that antagonized the vasopressor and sialagogic responses.

Cocaine, in doses comparable to those of imipramine or methylphenidate, affected only the pressor response to McN-A-343. The

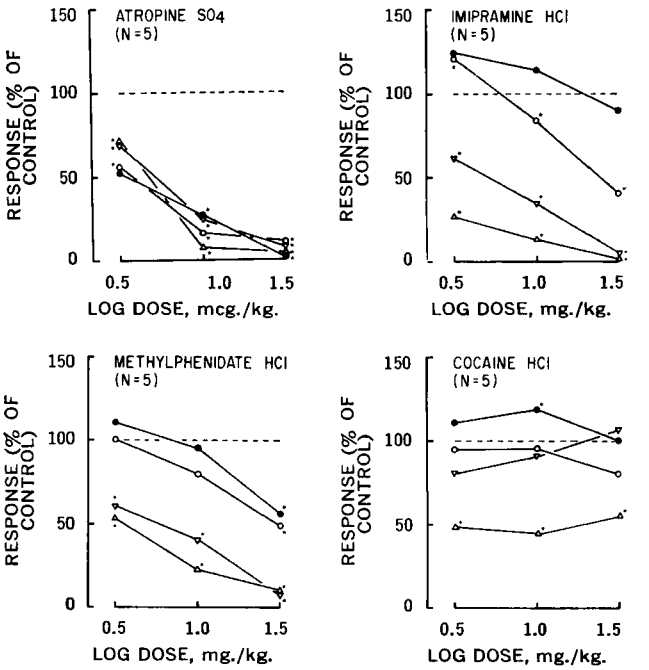

Figure 1—*Anticholinergic activities of atropine, imipramine, methylphenidate, and cocaine. Cholinergic responses are:* ○, *vasodepressor effect from acetylcholine (0.5 mcg./kg., i.v.);* ●, *vasodepressor;* △, *vasopressor;* ▽, *sialagogic effect from McN-A-343 (0.1 mg./kg., i.v.).* * = *significant difference from control at the 95% level (Dunnett's* t *test).*

antagonism was moderate and was not dose related. The depressor response to McN-A-343 was enhanced by the low doses of cocaine.

DISCUSSION

The results of this study indicate postsynaptic anticholinergic activities of imipramine and methylphenidate. In spite of the relatively low potency against the vasodepressor response of either acetylcholine or McN-A-343, a greater effect was observed in the vasopressor and sialagogic responses of McN-A-343. It is possible that imipramine and methylphenidate block the pressor response of McN-A-343 by inhibiting the release of norepinephrine from the sympathetic nerve ending since a high dose of imipramine was also reported to block the pressor response of dimethylphenylpiperazinium (DMPP) (1). However, the blockade of pressor response from McN-A-343 parallels the antisialagogic effect of imipramine and methylphenidate. This latter effect of McN-A-343 has been shown to be independent of the sympathetic ganglia and the adrenal medulla (8). The antisialagogic test with McN-A-343 is also a more selective test for anticholinergic activity since it is not blocked by cocaine which antagonizes the pressor response of McN-A-343 and tyramine.

It is noteworthy that atropine did not show the selective antagonism among the muscarinic responses as did imipramine and methylphenidate. This difference serves to illustrate the importance of selecting the animals test system which is most pertinent in the clinical use of drugs.

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Determination of Total Steroid Bases in *Solanum* Species

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Abstract □ A method is described whereby relatively small samples of leaves or berries may be screened for their alkaloid content. The glycoside is extracted by the usual methods and the partially pure product hydrolyzed to the aglycone. The aglycone is complexed with methyl orange and the colored complex extracted into chloroform and determined colorimetrically using solasodine as a standard. The colored complex obeys Beer's law in concentration from 10–120 mcg. in 5 ml. chloroform and has its maximum absorption at 420 mμ. The identity of the individual bases present may be determined by other methods such as chromatography.

Keyphrases □ Solasodine determination—*Solanum sodomaeum* and *S. laciniatum* □ Colorimetric analysis—spectrophotometer □ Methyl orange—color reagent

The steroid bases of the solasodine group occur naturally as the glycoside usually containing three sugars. On hydrolysis the glycosides yield the steroid alkaloid in the aglycone form. For example, the glycoside solanine yields solasodine and glucose, galactose, and rhamnose.

Alkaloid content of this material is usually determined by extraction of the dried material using continuous extraction apparatus, removal of the solvent, and precipitation of the bases by ammonia, followed by dissolving in acid, reprecipitation, drying, and weighing of the crude base. The resultant product is still impure and needs further purification by crystallization from an EtOH–water mixture. As some alkaloid is left in the mother liquor, this involves some losses and the determination is consequently inaccurate.

From a literature review of analytical methods for the determination of these bases the following methods have been noted.

Ruzhentseva and Tubina (1) use 4-hr. extraction by 5% acetic acid and precipitation by NH_4OH, 2-hr. drying and a further 2-hr. extraction by MeOH, and final potentiometric titration with 0.1 *N* HCl.

Wierzchowski (2) applies extraction by dilute acetic acid, precipitation by ammonia, solution in EtOH, and color formation using antimony chloride in concentrated HCl.

Balcar and Zalecka (3) use acetic acid extraction, hydrolysis, neutralization, and complex formation with bromothymol blue at pH 8.0 followed by colorimetric estimation.

It was observed that solasodine forms, with methyl orange, a yellow-colored complex which is soluble in chloroform, but at the same time the unhydrolyzed glycoside is not complexed. This was investigated further and it was established that the intensity of