Brit. J. Pharmacol. (1955), **10**, 147.

ACTION OF PROGUANIL AND ITS METABOLITE ON NEUROMUSCULAR AND SYNAPTIC TRANSMISSION

BY

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(RECEIVED APRIL 6, 1954)

The toxicity of the antimalarial drug proguanil (" Paludrine ") varies with its route of administration, but it is relatively low (Curd, Davey and Rose, 1945). Sometimes death ensues rapidly, sometimes after a few days (Butler, Davey, and Spinks, 1947). Such delayed toxicity has been attributed to its effect on cholinesterase (pseudocholinesterase of the blood serum, benzoylcholinesterase of the guinea-pig liver: Blaschko, Chou and Wajda, 1947) and is increased by neostigmine (Vane, 1949). Vane has shown that proguanil competes with acetylcholine, blocking its action on isolated rabbit auricle and guinea-pig ileum, inhibiting transmission in the phrenic-diaphragm preparation of the rat and in the sciatic-gastrocnemius preparation of the cat. In the rectus abdominis of the frog small doses of proguanil enhanced the actions of acetylcholine, large doses decreased the response, and still larger doses provoked a contracture. Hawking and Perry (1948) found that proguanil only affected *Plasmodium, in vitro,* in the presence of liver slices, suggesting that an active metabolite has to be formed. Crowter and Levi (1953) showed that *in vivo* the antimalarial action is due to 4:6-diamino-1-*para*chlorophenyl - 1:2 - dihydro - 2:2 - dimethyl-1:3:5-triazine—hereafter called triazine.

It seemed desirable to investigate further the actions of proguanil and triazine on neuromuscular transmission, and this paper describes such experiments in cats, dogs and rats. The actions of proguanil on the frog rectus abdominis, and on synaptic transmission in the superior cervical ganglion of the cat, have also been investigated.

METHODS

For the studies on neuromuscular transmission, cats and rats were anaesthetized with allobarbitone (Dial) and dogs with chloralose. The contractions of the tibialis anterior and the soleus in cats were induced by stimulation of the sciatic nerve, with ten supramaximal stimuli per minute (pulse duration, 1.0 msec.), from a Copeland stimulator (1951). In dogs, the movements of tibialis anterior and gastrocnemius were recorded; in rats, only the gastrocnemius. For observations on synaptic transmission in cats the cervical sympathetic was separated from the vagus and ligated below the point of stimulation. The contractions of the nictitating membrane were recorded in the usual way in response to supramaximal stimuli at 50 or 100/min. (pulse duration, 1.0 msec.) during which the drugs were usually injected intravenously. Sometimes the drugs were injected into the common carotid artery, all branches of which had been tied off except those supplying the superior cervical ganglion.

Arterial blood pressure was recorded in the cat and dog as a help in assessing the animal's condition. Artificial respiration was employed only in respiratory failure.

RESULTS

Proguanil

Action on Neuromuscular Transmission.—Intravenous injections of proguanil (5 to 10 mg./kg.) inhibit neuromuscular transmission in the cat. The inhibitions are more marked in soleus than in tibialis, and increase in intensity and duration on repetition of the injections (Fig. 1). Similar effects are found at the same dose-level in the dog and rat. Recovery from the block is delayed by decamethonium iodide (10 μg./kg.) and by eserine (400 μg./kg.). Where the decamethonium precedes the proguanil, the proguanil first increases the block, but thereafter there is a rapid restoration of transmission (Fig. 2). If the block is first developed under tubocurarine proguanil intensifies it and there is no such rapid recovery of transmission (Fig. 3, lower record). Responses to proguanil appear enhanced where transmission has recovered from block by either decamethonium (Fig. 3, upper record) or tubocurarine (Fig. 3, lower record), but these effects are difficult to evaluate because of the sensitizing or cumulative effects already referred to. When proguanil is given first, subsequent responses to decamethonium appear to be reduced and those to tubocurarine increased.

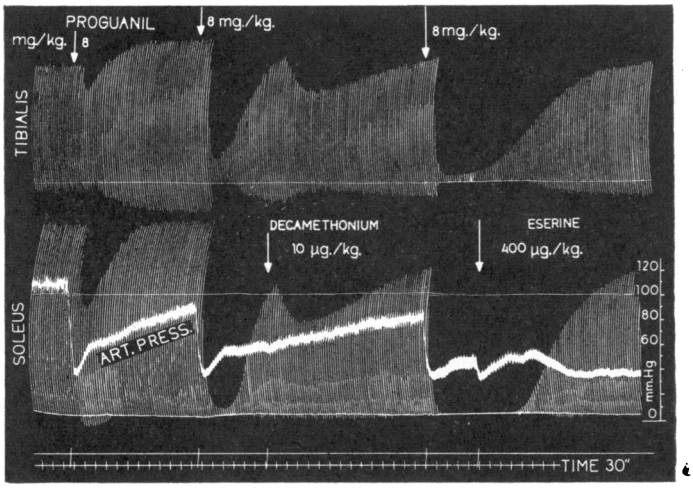

FIG. 1.—Cat (allobarbitone). Simultaneous records of contractions of the tibialis anterior and soleus muscle in response to single maximal shocks at 10/min. to the sciatic nerve. Record of blood pressure. From left to right, at arrows, i.v. 8 mg./kg. proguanil (twice); 10 μg./kg. decamethonium iodide; 8 mg./kg. proguanil; 400 μg./kg. eserine. Shows block of neuromuscular transmission by proguanil, and the delay in recovery produced by decamethonium and by eserine.

Although no evidence of a depolarizing action of proguanil on the synapses was found, these experiments gave some further evidence that proguanil and its metabolite triazine could act as competitive inhibitors. While the effect on the nictitating membrane appears to be brief, the responses to subsequent stimuli are diminished, reaching a minimum in some twenty minutes (Fig. 5B). Fig. 5C shows that faradization of the sympathetic chain (15 stim./sec.) rapidly restores normal transmission, and thereafter a further injection of proguanil has a two-stage action on the membrane. The usual initial block is followed, after partial recovery, by a more prolonged inhibition. This prolonged and delayed effect can be explained by the change in the molecule into its more active metabolite triazine. The biphasic character of the response does not appear as far as the blood pressure is concerned because the hypotensive action of

Because of the transitory nature of proguanil block, it is difficult to be dogmatic about its modification by anticholinesterases. In Fig. 1 eserine appears to prolong a block rather than diminish it, yet in other experiments eserine has appeared to reduce the cumulative effects of proguanil. In the cat, neostigmine is no better as an antagonist than eserine, but in the rat, as in Fig. 3, two injections of neostigmine relieve blocking, as does adrenaline.

Action on Synaptic Transmission.—The enhancement of a decamethonium block which precedes proguanil antagonism suggests that this antimalarial has a depolarizing as well as a curarimimetic action; but no evidence to support this has been obtained from a study of its actions on sympathetic synapses. In Fig. 4A the effect of increasing doses of proguanil on contractions produced by 50 stim./min. is shown between reference doses of ACh and adrenaline and stimulation at various frequencies (see legend). The proguanil response increases with the dose and is purely depressant, 5 mg./kg. producing a 60% inhibition. The sensitivities of the sympathetic synapse and the neuromuscular end plate would seem to be of the same order. Fig. 4B shows similar inhibitions of synaptic transmission with intra-ganglionic injections of proguanil. Here 50 μg. has almost as much effect as 1 mg./kg. i.v., and 250 μg. produces a very pronounced though brief inhibition.

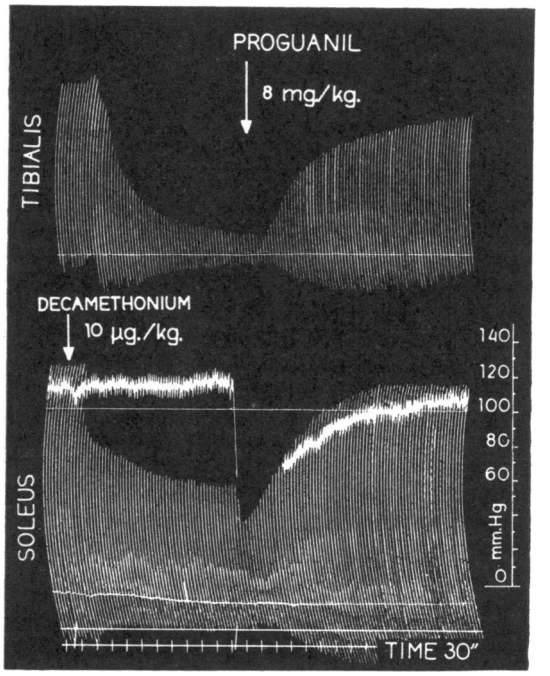

FIG. 2.—Similar preparation to that in Fig. 1. From left to right, at arrows, 10 μg./kg. decamethonium iodide; 8 mg./kg. proguanil. Shows that proguanil after decamethonium first increases block and then promotes restoration of neuromuscular transmission.

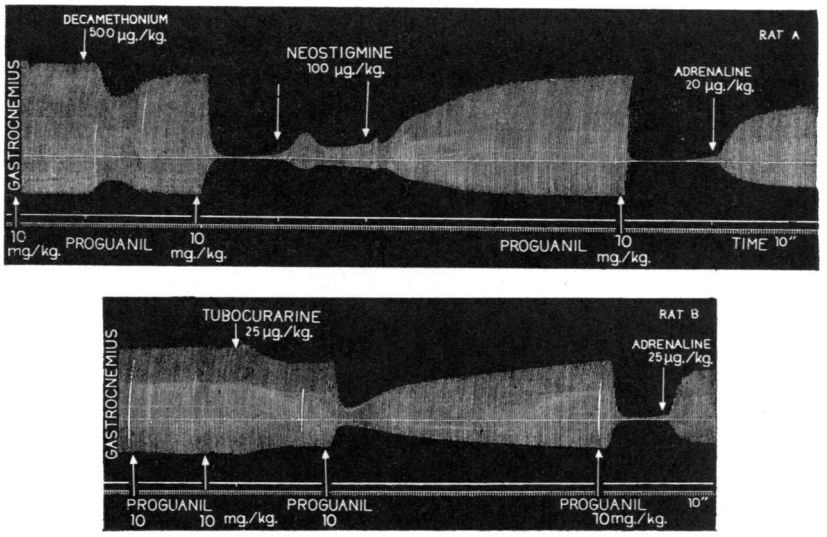

FIG. 3.—Rats (allobarbitone). Contractions of gastrocnemius induced by stimulation of the sciatic nerve (single maximal shocks at 10/min.). From left to right: upper record (rat A), 10 mg./kg. proguanil; 500 µg./kg. decamethonium iodide; 10 mg./kg. proguanil; 100 µg./kg. neostigmine (twice); 10 mg./kg. proguanil; 20 µg./kg. adrenaline. Lower record (rat B), 10 mg./kg. proguanil (twice); 25 µg./kg. tubocurarine; 10 mg./kg. proguanil (twice); 25 µg./kg. adrenaline. Shows enhancement of proguanil blocking action after recovery from decamethonium (upper record) and from tubocurarine (lower record) block.

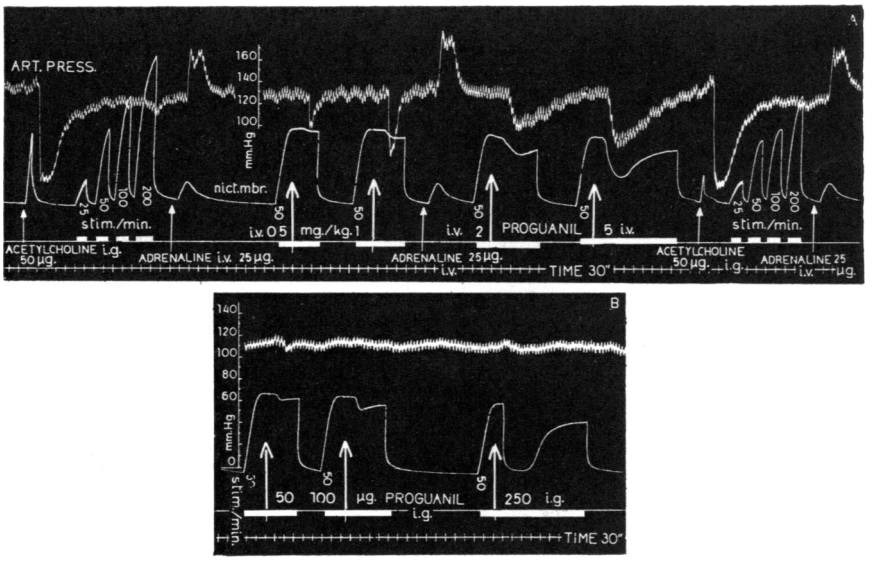

FIG. 4.—Cat (allobarbitone). Simultaneous records of blood pressure and nictitating membrane. From left to right: A (upper tracing), intra-ganglionic (i.g.) injection of 50 µg. acetylcholine; stimulations of the cervical sympathetic nerve (at 25, 50, 100, and 200/min.); i.v. injection of 25 µg. adrenaline. During successive stimulations at 50/min., 0.5, 1.0, 2, and 5 mg./kg. proguanil i.v. Between 2nd and 3rd proguanil injections, 25 µg. adrenaline i.v. Acetylcholine 50 µg. i.g. Stimulations of cervical sympathetic; adrenaline 25 µg. i.v. B (lower record), successive i.g. injections of 50, 100, and 250 µg. proguanil during stimulation of cervical sympathetic. Shows that proguanil blocks synaptic transmission both by intravenous (i.v.) and intra-ganglionic (i.g.) injection. This compound does not modify the adrenaline effect but lowers the stimulating action of ACh.

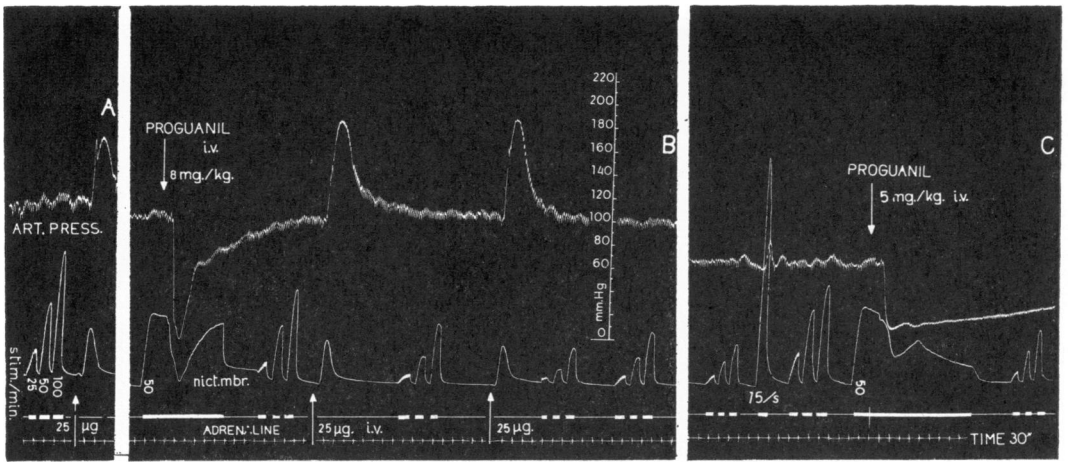

FIG. 5.—Cat (allobarbitone). Simultaneous record of blood pressure and nictitating membrane. Intravenous injections only. A—normal reactions of the cat: cervical sympathetic stimulations at 25, 50, and 100/min. respectively; 25 µg. adrenaline at arrow. B—during stimulation of cervical sympathetic (50/min.), 8 mg./kg. proguanil: a series of stimulations and of adrenaline injections (25 µg.). C—stimulations of the cervical sympathetic; faradization of the chain (15/sec.); stimulations of the chain. During stimulation at 50/min., 5 mg./kg. proguanil. Stimulations of the chain. Shows that proguanil action on synaptic transmission seems to be biphasic: a first transitory and short action is followed by a much longer inhibition. Faradization of the sympathetic chain restores transmission.

proguanil is much stronger than that of its metabolite.

Triazine

Qualitatively triazine acts as proguanil does, but quantitatively both neuromuscular and synaptic transmission are more affected by the metabolite: it is also more cumulative, for after repeated intravenous injections the response to 100 µg./kg. may equal the original response to 5 mg./kg.

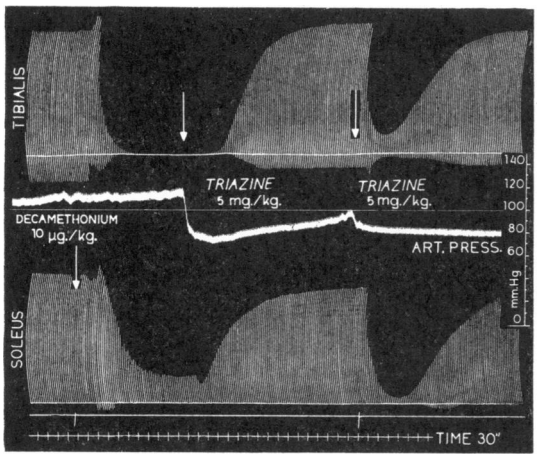

FIG. 6.—Similar preparation to that of Fig. 1. From left to right: 10 µg./kg. decamethonium iodide; 5 mg./kg. triazine (twice). Shows that triazine antagonizes blocking action of decamethonium.

Fig. 6 demonstrates its striking antagonism of decamethonium, while Fig. 7A and 7B give evidence of its effects on the synapse with one twentieth to one tenth of the dose required with proguanil. Neither when given intravenously, as in Fig. 7A, nor when injected into the ganglion through the ligated carotid, is there any apparent modification by triazine of the response of the nictitating membrane to adrenaline.

Rectus Abdominis of Frog.—Vane's observations (1949) on the actions of proguanil on the rectus muscle have been confirmed. This compound increases the action of ACh in low concentrations ($<10^{-6}$), but inhibits contraction at 10^{-5} and induces a contracture at 10^{-4}. The frog rectus, however, unlike mammalian tissues, is less sensitive to triazine than to proguanil. Triazine, 10^{-5}, inhibits the action of ACh 10^{-6} but not 10^{-5}. With ACh 10^{-5} plus triazine 2×10^{-4}, the muscle first contracts then relaxes and is insensitive to a new dose of ACh 10^{-5}, but rapidly becomes sensitive after washing. Even 2×10^{-4} triazine does not produce such contractures as were found with proguanil.

DISCUSSION

The evidence for competitive inhibition of neuromuscular transmission by proguanil and its metabolite is substantial. Thus their inhibitory action is not preceded by any increased muscle tonus, or increased response, as is usual with

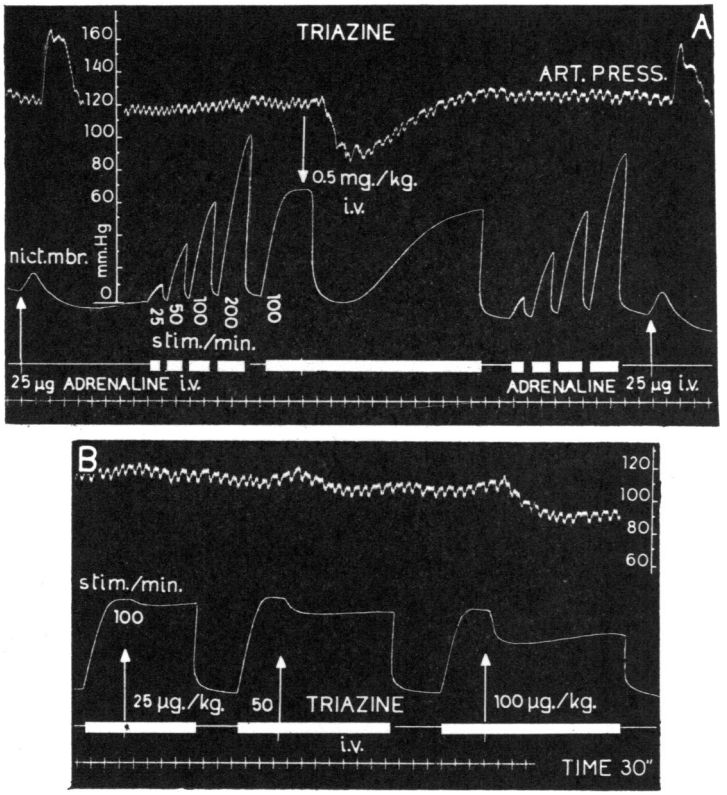

FIG. 7.—Similar preparation to that of Fig. 5. A—from left to right: 25 µg. adrenaline, stimulations of the cervical sympathetic chain (25, 50, 100, 200/min. maximal single shocks). During the stimulation (100/min.), intravenous injection of 0.5 mg./kg. triazine. Stimulations of the chain. 25 µg. adrenaline. B—triazine (25, 50, and 100 µg./kg. i.v.) during cervical sympathetic stimulation (100/min.). Shows that small doses of triazine (i.v.) inhibit synaptic transmission. Compare the doses with Fig. 4A.

decamethonium and other depolarizing blocking agents; they antagonize decamethonium and enhance the response to tubocurarine; their own blocking actions are reduced by adrenaline and by neostigmine; they are more active on soleus than on tibialis, and act at about the same dose level in cat, dog and rat. Eserine antagonism is, however, very slight if it exists, and the transitory additive effects of these substances on decamethonium block of neuromuscular transmission are difficult to interpret. No evidence of stimulation before the blocking of synaptic transmission has been found, but this does not rule out some possible depolarizing action on the motor end plate. It seems unlikely that the decamethonium enhancement is due to histamine release, for triazine gives no such depression of blood pressure as is seen with proguanil; moreover the recognized histamine-liberators seem to antagonize rather than to sum with decamethonium (Dallemagne and Philippot, 1953). It seems more reasonable to assume that it is the competitive inhibitory component of the action of decamethonium (Zaimis, 1952) that is enhanced by proguanil and triazine; this is the more likely since the additive effects are more marked on soleus than tibialis, soleus being more affected by competitive blocking drugs. Thus the actions of proguanil and triazine on the motor end plate are comparable to those of the higher homologues of the alkyl-trimethyl ammonium salts (Dallemagne and Philippot, 1951).

Triazine is somewhat more efficient than proguanil in blocking the motor end plate, and markedly more so on synaptic transmission. If, as has been suggested, the prolonged biphasic response of the nictitating membrane recorded in Fig. 5C is due to the conversion of proguanil to triazine, the cyclization of the biguanide chain must take place very rapidly in the body.

These results make it difficult to explain Vane's (1949) observations on the enhancement of the toxicity of proguanil by neostigmine—as an antagonist at the neuromuscular junction neostigmine might be expected to reduce the toxicity of proguanil—but this toxicity may not be due to its depression of neuromuscular transmission and hence of respiration.

SUMMARY

1. Proguanil and its metabolite, triazine, are competitive inhibitors of neuromuscular and synaptic transmission.

2. The metabolite is more active than proguanil in inhibiting neuromuscular transmission and much more active in inhibiting synaptic transmission.

3. Proguanil is rapidly transformed into triazine in the organism.

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