The Effect of Diphenylhydantoin on the Response to Accelerator Nerve Stimulation[1,2] (34775)

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In 1950 it was reported that diphenylhydantoin (Dilantin) was effective in suppressing arrhythmias resulting from experimentally induced myocardial infarction (1). Since this report, a number of investigators have demonstrated that diphenylhydantoin is effective in abolishing a large variety of ventricular arrhythmias (cf. 2, and 3 for references). It has been reported to be particularly effective against arrhythmias induced by digitalis materials (4–6).

In a series of reports beginning in 1961, Roberts and his co-workers (7–9) drew attention to the possibility that the capacity of digitalis materials to induce ventricular arrhythmias is related, at least in part, to the action of these drugs which increases activity in adrenergic nerves innervating the heart. It was demonstrated that pharmacologic procedures which diminish adrenergic nervous activity also reduced the capacity of digitalis to induce arrhythmias. It has also been shown that interruption of adrenergic pathways by surgical means increases the dose of ouabain required to produce death in dogs and cats (10, 11). In addition, total cardiac denervation has been reported to diminish the capacity of digitalis to induce arrhythmias (12). It is noteworthy that beta-adrenergic blocking agents which inhibit digitalis-induced arrhythmias also cause neurodepression, while those which are not antiarrhythmic agents do not affect neural activity (13). These observations taken together with those demonstrating that digitalis causes convulsions (14), stimulates the vomiting (15) and the respiratory center (16) strongly suggest that digitalis has the capacity to increase activity in neural structures innervating the heart.

The fact that neurodepression diminishes the capacity of digitalis materials to produce arrhythmia may also relate to the antidigitalis action of diphenylhydantoin. The anticonvulsant properties of this agent are well-known and its effects on peripheral nerve excitation and ganglionic transmission have been described (17, 18). In addition, diphenylhydantoin depresses motor nerve terminal activity in the cat soleus nerve preparation (19).

The present investigation was initiated to further explore the relationship between neural depression and the antiarrhythmic activity of diphenylhydantoin. In particular, to determine whether diphenylhydantoin diminishes adrenergic nervous activity in dose ranges which have been reported to antagonize digitalis-induced arrhythmias. The data show that diphenylhydantoin reduces both pre- and postganglionic-induced acceleration of the heart rate in doses which do not affect reactivity of the adrenergic receptor to isoproterenol.

Methods. All experiments were performed in cats anesthetized with α-chloralose, 80–100 mg/kg, administered intravenously. The trachea was cannulated and the animals were artifically respirated with oxygen; the respirator was set to breathe the animal at a rate of 18/min. Blood samples (0.5 ml) were withdrawn through a polyethylene catheter inserted into the right femoral artery. Samples were tested every 5–10 min throughout the experiment for pH, pO_2, and pCO_2 using a physiological gas analyzer (Beckman, Model 160). The blood samples were returned to the animal after each test and if necessary,

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the volume and oxygen content (40–100%) of the inspired air were adjusted to maintain blood pH and pO_2 at control levels, (pH 7.4–7.5; pO_2 200–400 mm Hg). P CO_2 prior to artificial respiration was usually between 20–30 mm Hg and during artificial respiration, between 10–20 mm Hg.

Blood pressure was recorded by inserting a saline-filled catheter into the left femoral artery which in turn was connected to a Statham pressure transducer (P23Gb); the output of the transducer was recorded on a two-channel polygraph (Offner).

All injections or infusions of drug solutions were made through a catheter placed in the right femoral vein.

Heart rate was determined by counting each QRS complex of the EKG (usually lead II) through a specially designed signal discriminator whose output was recorded by a tachometer. The tachometer output was registered on the second channel of the polygraph. Changes in rhythm were determined by monitoring the EKG on a Tektronix Oscilloscope (502).

The right sympathetic trunk was isolated and prepared for stimulation in the following way: The right thoracic cavity was opened by resecting the clavicle and the first five ribs. The sympathetic chain was separated out to the level of T4 or T5 at which point the nerve was tied and crushed proximal to the tie. The right stellate ganglion was identified and the branch giving marked acceleration of the heart rate on electrical stimulation was prepared for stimulation during the experiment. The pre- and postganglionic fibers were stimulated through platinum electrodes using an appropriate Grass stimulator (S4). The intensity of stimulation was selected so that during a 40-sec stimulation period, the heart rate would increase 30–40 beats/min. Stimuli of 5–10 V at frequencies of 1–5 impulses/sec of 0.5 msec duration applied either to the pre- or postganglionic fibers were usually sufficient to produce this magnitude of acceleration.

Atropine (2 mg/kg i.v.) was administered before nerve stimulation was instituted. Gallamine (2 mg/kg i.v.) was administered periodically throughout the experiment to prevent muscle movement which might cause the tachometer to give erroneous results. Isoproterenol (Isuprel) was injected in doses which produced acceleration of a magnitude similar to that caused by nerve stimulation, *i.e.*, 30–40 beats/min. It was found that 0.05–0.2 μg/kg were usually sufficient for this purpose. Diphenylhydantoin (DPH) was administered at a rate of 2 mg/kg/min in distilled water made alkaline (pH 11) by the addition of sodium hydroxide. The solution was made freshly before each experiment. The small doses used in these experiments (5 and 10 mg/kg) have been reported to approximate the minimally effective anticonvulsant dose in cats (18, 20). The minimal neurotoxic dose of DPH in cats was reported to be approximately 40 mg/kg.

The experimental sequence was arranged in the following way: The preganglionic fibers were stimulated first, followed in 5 min by postganglionic nerve stimulation which was then followed in 5 min by the injection of isoproterenol. Five minutes after the injection of isoproterenol, the sequence was repeated again. This pattern was continued throughout the experiment. The test drug was not administered until in three consecutive sequences the acceleration produced by nerve and drug stimulation was reproducible, *i.e.*, within three beats of each other. The standard error of the mean is indicated after each average response and the Student t test was used to test statistical significance of the difference between means.

Results. Effect of small doses of DPH on acceleration induced by nerve stimulation or by isoproterenol. In doses of 5 mg/kg DPH did not significantly affect the acceleration produced either by pre- or postganglionic stimulation. Increasing the dose of 10 mg/kg resulted in a significant reduction of neurally-induced acceleration (Fig. 1). Immediately after the injection of DPH was completed preganglionic nerve stimulation caused acceleration which was only on the average 74.8 $\pm$ 7.8% of control ($p<.05$). This effect was short-lived and 15 min after the injection of DPH the depression of the response to preganglionic stimulation was not statistically significant (88.3 $\pm$ 5.8% control; $p>.05$).

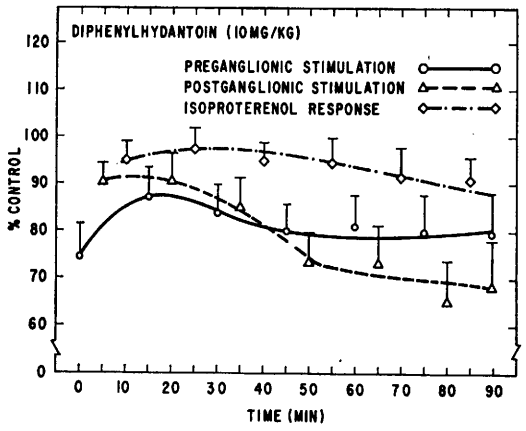

FIG. 1. The effect of diphenylhydantoin (10 mg/kg) on acceleration induced by pre- and postganglionic nerve stimulation and by isoproterenol. DPH was administered intravenously at a rate of 2 mg/kg/min; total duration of injection was 5 min. Zero time refers to the end of the DPH infusion. The points depicting the response to nerve stimulation (pre- and postganglionic stimulation) and isoproterenol represent the average of 5–14 observations. The magnitude of acceleration induced by either nerve or drug stimulation prior to DPH administration ranged between 30 and 40 beats per minute. The vertical bars represent the standard error of the mean. (See *Methods* for description of experimental design).

Soon after, however, diminution in the response was observed again, and 45 min after DPH preganglionic stimulation caused an acceleration of only 79.7 ± 7% of control ($p < .05$). This effect persisted for at least another 45 min since in three instances in which observations were made up to 2.5 hr after DPH, no change in the level of depression was noted.

Depression of the response to postganglionic stimulation did not appear until 50 min after the injection of DPH; the degree of depression was similar in magnitude to that of the preganglionic response (Fig. 1). The duration of this depression also closely paralleled that observed with preganglionic stimulation. Thus, in three experiments 2.5 hr after the administration of DPH, the postganglionic response was still only 70% of control.

Isoproterenol-induced acceleration was not significantly depressed by DPH (10 mg/kg) at any time during the experiment (Fig. 1). It is noteworthy that even during the later stages of the experiment when both pre- and postganglionic nerve stimulation were significantly depressed by DPH, the acceleration induced by isoproterenol was not significantly different from the controls ($p > .05$).

Other effects of small doses of DPH (10 mg/kg). The average heart rate in 14 animals just prior to the injection of DPH was 181 ± 8.2 beats/min. After the injection of DPH was completed, the heart rate was 89.4 ± 1.4% of control ($p < .05$), and recovery to control levels occurred approximately 30 min later. The average blood pressure in 15 animals just prior to the injection of DPH was 164 ± 9.1 mm Hg. After the drug, blood pressure was slightly lowered and it was significantly depressed (89.6 ± 1.90% of control; $p < .05$), only immediately after the injection of DPH.

In addition to the effects on heart rate and blood pressure, DPH in four animals caused a transitory rise in blood pH (7.5–7.55). This was associated with an increase in the effect of nerve stimulation and isoproterenol. These effects were not included in the calculations of the average responses after DPH was administered since they were not directly related to drug action but rather to changes in pH probably caused by the alkaline diluent. The administration of just the diluent to three animals caused in two of them an increase in pH (7.5–7.52) and in the response to nerve stimulation and isoproterenol which lasted for 15–20 min.

It should be emphasized that the results of these experiments could have been altered not only by changes in pH due to diluent but also by changes in pH and pO_2 occurring spontaneously during the experiment. Frequent monitoring of blood pH and gases prevented this since when a change was noted appropriate adjustment in the volume or the O_2 content of the inspired air was made to return pH and pO_2 to control levels. Although no systematic study of the effect of pH on nerve and drug-induced acceleration was performed it was observed that when the pH measured above 7.5, the acceleration induced by either isoproterenol or nerve stimulation was enhanced while when the pH fell

below 7.4, the acceleration induced by the nerve or isoproterenol was less than that noted at normal pH.

Effect of large doses of DPH on acceleration induced by nerve stimulation or by isoproterenol. Increasing the dose of DPH from 10 mg/kg to 20 mg/kg produced marked changes in the capacity of the drug to depress nerve stimulation. The response to pre- and postganglionic stimulation was depressed to the same degree, *i.e.*, to 36.7 ± 11.1 and 37.5 ± 13.1% of control, respectively (Fig. 2). These effects occurred within the first 5 min after the injection of DPH was terminated. This degree of depression persisted throughout the experiment. Indeed, in three experiments in which observations were made up to 2.5 hr after the injection of DPH, the depression of pre- and postganglionic acceleration was of the same order of magnitude as that seen earlier after drug administration.

The large dose of DPH (20 mg/kg) also caused greater depression of the response to isoproterenol than that produced by the smaller doses but this effect did not prove to be statistically significant until 40 min (Fig. 2)

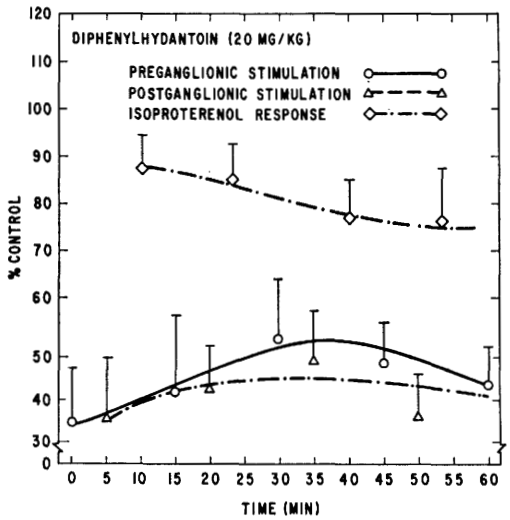

FIG. 2. Effect of diphenylhydantoin (20 mg/kg) on acceleration induced by pre- and postganglionic stimulation and by isoproterenol. Conditions same as described in legend to Fig. 1 except that the number of observations at each point ranged between 4 and 7. Total duration of injection was 10 min.

after the injection of DPH ($p < .05$). Even at the time when depression proved to be statistically significant (Fig. 2), *i.e.*, 40 and 55 min after the drug, the magnitude of the decrease in the response (24.0 and 23.0% reduction, respectively) was not comparable to that of the nerve response. Thus, the response to preganglionic stimulation was reduced 51.2% and 56.4% 45 and 60 min after DPH, respectively, while the effect of postganglionic stimulation was reduced 50.9% and 64.5% 35 and 50 min after DPH, respectively. In three animals in which the experiments were conducted up to 2.5 hr after DPH, the degree of depression in the response to the nerve or isoproterenol observed after 60 min stayed at this level throughout the remainder of the experiment.

In four animals, 30–40 mg/kg were injected but since the blood pressure and heart rate fell to extremely low levels and in the one instance death resulted, the effect of these large doses of DPH on nerve or isoproterenol-induced acceleration was not explored.

Other effects of the larger dose of DPH (20 mg/kg). The effect of the larger dose of DPH (20 mg/kg) on blood pressure was relatively short lived. The average blood pressure in seven animals was 168 ± 7.8 mm Hg but after DPH it was only 87.9 ± 2.5% of control ($p < .05$). Five minutes after DPH, the decrease in blood pressure was not statistically significant ($p > .05$). The heart rate, however, remained significantly depressed ($p < .05$) throughout the experiment (60 min), the greatest decrease occurring within the first 5 min of the injection. The average control heart in seven animals was 188 ± 5.6 beats/min but immediately after DPH it fell to 164 ± 4.2 beats/min. Ten minutes after DPH, the heart rate was approximately 93% of control and it remained at about this level for the remainder of the experiment.

The same type of effect as that produced by lower doses on pH was also observed with the larger dose (20 mg/kg), *i.e.*, in three animals the pH increased to 7.5–7.6. Changes in response to nerve stimulation or to isoproterenol which occurred before the pH could be brought back to normal were not included

in the calculation of the average responses.

Discussion. The results of these investigations clearly demonstrate that DPH depresses nerve-induced acceleration and that this effect is not a result of receptor blockade by the drug but rather due to a depressant action on neural structures *per se*. Thus, at the time acceleration induced by pre- and postganglionic stimulation was depressed to levels of approximately 40% of control (Fig. 2) the response to isoproterenol was not significantly affected. Only later on in the experiment and only after 20 mg/kg was the response to isoproterenol significantly reduced. Even at this point, it was reduced about 25% whereas the responses to nerve stimulation were reduced by at least 50%.

The blockade of isoproterenol acceleration developing 40 min after the injection of 20 mg/kg of DPH is not in agreement with the results of Raines and Levitt (21) which showed that DPH does not influence the response to isoproterenol. It is possible that the differences in rate of injection and length of the experiments may account for the differences in the results, *i.e.,* Raines and Levitt (21) infused DPH at a slower rate than that used in the present experiment. Furthermore, their experiments were performed in cats with the spinal cord transected at C1. It should be emphasized, however, that the failure of the large dose of DPH to produce early depression of the isoproterenol response and the lack of any effect with smaller dose is in agreement with the results of Raines and Levitt (21) and those of Strauss *et al.,* (3) obtained in isolated rabbit atria. In any case, the data show that depression of nerve-induced acceleration by DPH is not due to the blockade of beta adrenergic receptors in the heart.

The action of DPH to depress accelerator nerve responses agrees with the observations that synaptic transmission in the stellate ganglion is diminished by this agent (18). Indeed, it was demonstrated in cats in the same dose range (10–30 mg/kg) used in the present study that DPH increased transmission failure and reduced posttetanic potentiation. Nevertheless, it should be emphasized that this inhibitory effect on ganglionic transmission is not the sole explanation for the depressant action of DPH on nerve-induced acceleration. The effect of postganglionic stimulation was also reduced although at the lower dose (10 mg/kg) it required a longer period for it to develop and it was usually of a smaller order of magnitude than the reduction in preganglionic responses. However, after larger doses, the responses to pre- and postganglionic stimulation were depressed to a similar extent.

The effect of DPH on blood pressure did not seem to influence its depressant effect of nerve-induced acceleration. While blood pressure was significantly reduced even with the smaller doses (10 mg/kg) it was not usually depressed more than 15%. Furthermore, the depression was short-lived, lasting but 5 min even after 20 mg/kg was administered. Since the depression of nerve stimulation was of greater magnitude and longer lasting than the drug effect on blood pressure, it seems unlikely that the two actions are related.

The effect of DPH on heart rate was more prominent than on blood pressure lasting for longer periods of time. It is unlikely, however, that a relationship exists between the effect on heart rate and the effect on nerve stimulation. While after the larger dose of DPH (20 mg/kg) depression of the heart rate paralleled in time the depression of nerve stimulation, it did not closely follow the pattern of depression after the smaller dose (10 mg/kg). Thus, while significant depression of the heart rate was noted during the first 30 min after DPH administration, during the next 60 min when the response to both pre- and postganglionic stimulation was depressed, heart rate was not significantly affected. Furthermore, since the lowering of heart rate either by the small or large dose of DPH was not usually accompanied by a reduction in the acceleration produced by isoproterenol, the slowing of the heart rate *per se* is not responsible for the reduced capacity of the heart to accelerate after DPH.

The depressant action of DPH on the accelerator nerve occurred to prevent digitalis-induced arrthythmias in the cat (22). Thus, the results of the present investigation indicate that a significant reduction in adrenergic nervous activity is associated with the anti-

arrhythmic effects of DPH. It is conceivable that if digitalis induces arrhythmia, at least in part, by increasing adrenergic nervous activity, this effect of the glucoside would be diminished by DPH. In two recent reports, an excitatory effect on the adrenergic nervous activity by digitalis materials have been demonstrated (23, 24). Furthermore, it has been recently shown that after reserpine pretreatment, the capacity of the DPH to antagonize digitalis-induced arrhythmias is reduced (25). This supports the contention that part of the antiarrythmic effects of DPH is related to an action on adrenergic nervous activity. It is important to note, however, that after adrenergic nervous activity was abolished by reserpine, DPH was still effective against the more ominous types of arrhythmias produced by digitalis materials, *i.e.*, ventricular tachycardia and fibrillation. This indicates that digitalis and DPH exert actions directly on the heart independent of their effects on the adrenergic nervous system. In this regard, DPH has been shown to depress automaticity of pacemaker tissue and to increase conduction velocity (2, 6). It seems, therefore, that the capacity of DPH to antagonize digitalis-induced arrhythmias is related to its actions which affect nervous as well as muscle activity. Whether both of these actions are the basis for its effects against rhythm disturbances caused by other means is uncertain.

During the course of these experiments, it was noted that the effect of adrenergic nerve stimulation and isoproterenol injection varied if the blood pH was changed. While such influences have been reported for the action of catecholamines on the heart (26), apparently such studies in the case of the nerve have not been performed. Although the present study was not designed to explore these relationships, the results point to the necessity of controlling pH throughout the experiment. Indeed, that pH changes may occur spontaneously should not be surprising in view of the fact that the animals have their chest opened and they are artificially respirated.

Summary. Diphenylhydantoin (DPH) in doses as low as 10 mg/kg produces significant and long lasting depression of the accelerator response to pre- and postganglionic stimulation. The response to isoproterenol was not affected when neurally-induced acceleration was diminished. Only after the very large dose of DPH was the isoproterenol response diminished and it required a much longer period to develop than the reduction in the neural effect. It was concluded that diminution in nerve-induced acceleration by DPH is due to a neural depressant effect rather than an effect on beta-adrenergic receptors. There did not appear to be any correlation between the action of the drug to decrease blood pressure and heart rate and the action of the drug to depress the response to accelerator nerve stimulation. Since the neural depressant action occurred with doses which are also effective against digitalis-induced arrhythmia, this antiarrhythmic effect of DPH seems to be associated with depression of adrenergic nervous activity.

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