Pyrogallol and Vasopressin Tachyphylaxis

It is well established that repeated injections of vasopressin will result in a rapid loss of its ability to raise blood pressure. Because of this reproducible action vasopressin is frequently cited as a classical example of a drug displaying the characteristic of tachyphylaxis[1]. In spite of sporadic investigations, tachyphylaxis to vasopressin has not been adequately explained. WOODBURY[2] showed that ouabain could prevent the development of tachyphylaxis when given before vasopressin and could restore the pressor response after the establishment of tachyphylaxis. More recently, NASH[3] has reported that in the reserpinized dog, tachyphylaxis is greatly reduced. In the present report data are presented to show that pyrogallol (1, 2, 3-trihydroxybenzene), a catechol o-methyl transferase (COMT) enzyme inhibitor, can also prevent and interrupt tachyphylaxis to vasopressin.

In this study 42 dogs of either sex were anesthetized with pentobarbital sodium, 30 mg/kg body weight intravenously. Blood pressure by mercury manometer and respiration by Marey tambour were recorded on a kymograph. Constant injections of vasopressin, 0.3 U/kg body weight, were given every 30 min throughout the experiment. This dose schedule has been shown previously[4] to produce nearly complete loss of pressor effects within 3 to 4 doses.

In the first series of experiments pyrogallol was given after tachyphylaxis to the pressor response of vasopressin had developed. Doses of pyrogallol varied from 0.5 to 32 mg/kg and were given 3 to 5 min prior to the 5th and subsequent doses of vasopressin. Of the 24 dogs in this group, pyrogallol slightly increased the pressor responses to vasopressin in 5, brought the response back to about half the control in 10, and completely restored the original response in 9 dogs (Figure 1). In several of these latter animals pyrogallol was repeated just before additional doses of vasopressin and the pressor responses were well maintained. The initial injection in Figure 1 illustrates the triphasic blood pressure response that is sometimes obtained with vasopressin. This includes a depressor component that is thought to be due to coronary artery constriction. Tachyphylaxis apparently occurs to this depressor effect also, but pyrogallol only restored the pressor component.

In general, the higher doses of pyrogallol were likely to have more effect than smaller doses. Doses of 1–2 mg/kg were sometimes effective and sometimes not, 4 mg/kg were more predictable, and 8 mg/kg appeared to be an optimum amount. Doses greater than this usually failed to produce any greater response. A single dose of pyrogallol generally affected only that dose of vasopressin which immediately followed it, although in a few cases the next two doses were influenced.

In a separate group of animals, pyrogallol was given just before the second dose of vasopressin and repeated just before each succeeding dose. In this way it may be possible to prevent the development of tachyphylaxis or to greatly delay its appearance. Figure 2 shows an experiment in which the first and fourth responses to vasopressin were identical in amplitude.

A few experiments with isomers of pyrogallol suggest that 1, 2, 4-trihydroxybenzene has virtually no such effect while 1, 3, 5-trihydroxybenzene (phloroglycin) has some

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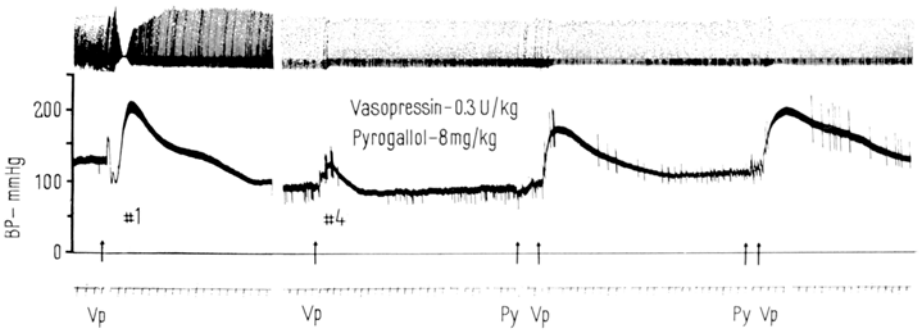

Fig. 1. Restoration by pyrogallol of the pressor response to vasopressin after tachyphylaxis had developed. Tracings from top to bottom: respiration, mean blood pressure, zero reference, and time in 1 min intervals. Pyrogallol was given just prior to the 5th and 6th doses of vasopressin.

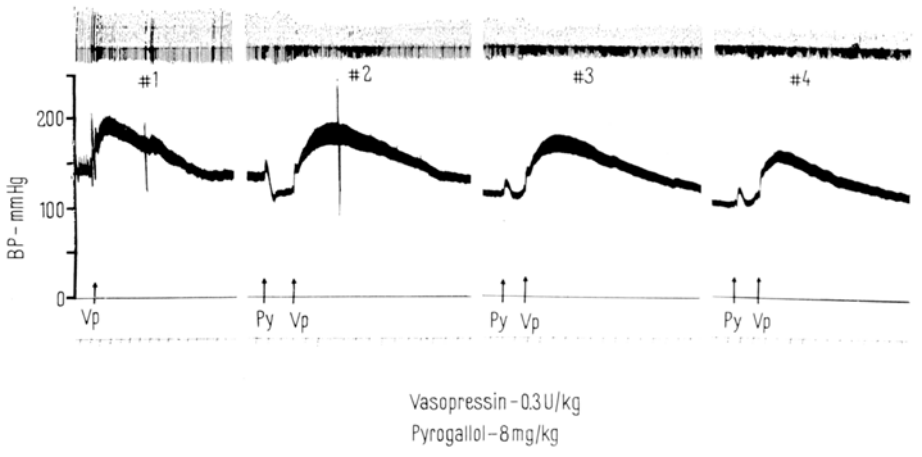

Fig. 2. Prevention of tachyphylaxis to vasopressin by pyrogallol. Tracings are the same as in Figure 1. Vasopressin was given every 30 min with the 2nd, 3rd, and 4th doses being preceded by pyrogallol. The pressor response to the 4th dose of vasopressin was the same as the first.

activity but much less than pyrogallol. The COMT inhibitor, catechol (1,2-dihydroxybenzene), did not appear to be active in this regard.

The finding that the blood pressure response to vasopressin is markedly affected by pyrogallol may have wide import because of the current interest in agents that affect the metabolism of catecholamines. It is now known that one of the major routes of degradation of epinephrine and norepinephrine is *via* o-methylation[5]. The enzyme involved, catechol o-methyl transferase, can be effectively inhibited by furnishing a competitive substrate, such as pyrogallol[6,7]. The presence of pyrogallol slows the destruction of epinephrine and norepinephrine and extends their duration of action[8,9]. However, vasopressin has long been classed as a direct-acting vasoconstrictor with a mechanism of action unrelated to catecholamines, and it was surprising, therefore, to find that a COMT inhibitor would modify its response. It is well known that adrenergic blocking agents do not prevent the pressor response to vasopressin, and NASH[3] has shown that complete depletion of catecholamines by reserpine pretreatment not only does not depress the response but actually increases it.

The data reported here might be interpreted as indicating an intimate relationship between catecholamines and vasopressin vascular effects. Support for this concept could be found in the work of GARDIER[10,11], who has demonstrated a relationship between the sympathetic nervous system and the effect of vasopressin on blood pressure. Other workers have implicated cyclic 3′,5′-AMP in the mechanism of action of both epinephrine and vasopressin[12,13].

In the present case, however, the weight of evidence opposes this view. In addition to the failure of adrenergic blockade or catecholamine depletion by reserpine to reduce the pressor action of vasopressin, it appears that certain other COMT inhibitors (e.g. catechol) do not have this action of pyrogallol. These data indicate that the COMT inhibiting action of pyrogallol is not the mechanism involved. The suggestion of a non-COMT inhibiting action is also supported by the recent work of HALMAGYI et al.[14], who have shown a pulmonary antispasmodic action of pyrogallol which is unrelated to COMT inhibition. Alternatively, it is suggested that pyrogallol is able to alter in some way the receptors for vasopressin or to increase the dissociation of vasopressin from its receptor complex in such a way as to permit repeated responses to occur. The basis for this action is obscure and requires further study[15,16].

Résumé. Le pyrogallol empêche le développement d'une tachyphylaxie à la vasopressine. Il restitue la réaction pressorique après l'apparition d'une tachyphylaxie. Cet effet ne semble pas être lié à l'action inhibitrice de cette substance sur l'enzyme transférase o-méthyl.

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[16] I thank Mrs. BETTE LITTLE for technical assistance.

The Effect of Isoxsuprine on the Motility Pattern of the Isolated Human Myometrium[1]

In a previous report[2] it was demonstrated that the action of prostaglandin on the isolated human non-pregnant myometrium could be affected by variations in the extracellular concentration of potassium. The normal effect of prostaglandin on the myometrium is that of inhibition. A decrease in the extra-cellular potassium concentration enhances the inhibitory response of human isolated myometrium to prostaglandin. However, the addition of prostaglandin to a myometrium placed in a potassium-rich bath fluid produces only a small inhibition of motility, or, in some instances, even induces a stimulation of the preparation.

In order to study the specificity of this effect of potassium on the reactivity pattern of human myometrium to prostaglandin, a similar study has been performed with isoxsuprine, a compound which also inhibits the spontaneous activity of the human myometrium[3].

The experimental conditions were the same as in the previous study, i.e. the motility of excised strips from the human uterus was studied *in vitro* using almost isometrical recordings. Four strips from each uterus were studied simultaneously, each strip mounted in separate 40 ml cuvettes in an organ bath. The bath fluid was a slightly modified Tyrode solution aerated with 5% CO_2 in O_2, pH being 7.35 ± 0.05. The active compound was always added to the bath diluted in 1 ml of a fluid with the same composition and temperature as the bath fluid. A continuous inflow of fresh Tyrode from below at a rate of 1.5 ml/min slowly washed away the added compound. This procedure was adopted since it gave stable experimental conditions and reproduceable results. For details the reader is referred to a previous communication[4].

In the present study 12 strips from three uteri all in late proliferative phase were used. The effect of isoxsuprine on the spontaneous motility of the isolated human myometrium under different extra-cellular potassium concentrations, 1–17 mEqv/l, was tested. The preparation used

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