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EFFECTS OF LONG-TERM ADMINISTRATION OF PYROGALLOL ON TISSUE CATECHOLAMINE LEVELS, MONOAMINE OXIDASE AND CATECHOL-O-METHYL-TRANSFERASE ACTIVITIES IN THE RAT

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Abstract—Experiments have been performed to obtain evidence that the altered levels of endogenous catecholamines produced by pyrogallol treatment may be regulated by an adaptive change in the activities of catechol-O-methyltransferase and monoamine oxidase.[5]

The effects of chronic administration of pyrogallol on the content of catecholamines and on the activity of catechol amine inactivating enzymes of rat organs have been studied. The catecholamine content in the brain and in the adrenals was not altered significantly by pyrogallol treatment. It was usually smaller in the heart of treated animals than in the controls. Catechol-O-methyltransferase activities in the liver, brain and heart remained unchanged or were slightly higher under chronic pyrogallol administration. The acute responsiveness of catechol-O-methyltransferase to pyrogallol injection was not modified by chronic treatment with pyrogallol. Monoamine oxidase activity was not influenced in the brain. From the third week of treatment it was increased in the heart and decreased in the liver.

No evidence for the occurrence of adaptive changes in the activities of catecholamine metabolizing enzymes was found, since no change or very small changes were observed in the endogenous catecholamine levels and in the activities of tissue monoamine oxidase and catechol-O-methyltransferase.

PYROGALLOL is known to be a potent inhibitor of catechol-O-methyltransferase (COMT).[1–3] Its inhibitory effects are of short duration *in vivo*. Crout[4] found that effective inhibition of the enzyme could be achieved for 30–60 min after a single dose of 200 mg/kg and that a repeated administration every 30 min was required for a more prolonged enzyme inhibition by a lower dose of 50 mg/kg. Most of the studies so far have dealt with the acute effects of pyrogallol. However, very interesting results about the effects of chronic administration of pyrogallol in rabbits and rats have been reported by Nukada *et al.*[5] These authors showed that in the rabbit excretion of O-methylated catecholamines decreased within 1 or 2 days after pyrogallol injection and increased then to normal values at the end of the third week. The changes in the excretion of vanillylmandelic acid showed the same tendency but were quantitatively less pronounced, whereas the excretion of catecholamines rose gradually during 3 weeks and remained higher than in control animals. In the rat, the same authors found that administration of pyrogallol slightly decreased hepatic monoamine oxidase

(MAO) and COMT activities until about the third week, but that continued treatment for 5 weeks significantly stimulated these activities. Finally, the authors suggested that the altered level of endogenous catecholamines may be regulated by an adaptive change in the activities of MAO and COMT.

The present study was undertaken to evaluate the effects of chronic administration of pyrogallol on these two enzymes and simultaneously on the content of catecholamines at the tissue level. In order to gain a better insight of correlations between these different effects, all experiments were performed with rats. Enzymatic activities were determined in brain, heart and liver. For catecholamine analysis, brain, heart and adrenals were used.

MATERIALS AND METHODS

Treatment of animals

Male rats, 150–180 g body wt. received daily subcutaneous injections of pyrogallol (50 mg pyrogallol purissimum, CIBA, kg body wt.). Pyrogallol (25 mg/ml) was dissolved freshly in physiological saline in bottles containing no more than 20 ml. Control rats received daily the same vol. of solvent. For the estimation of catecholamines and of MAO activity, the organs were removed 22–24 hr after the last injection. Determinations of COMT activity were carried out both 30 min and 22–24 hr after the last injection.

Extraction of catecholamines

(a) *Adrenals.* Pairs of adrenals were minced in glass homogenizers and extracted with 10% TCA. These extracts were found to be pure enough to permit fluorimetric determination directly, without adsorption on to alumina. A similar observation was made for perchloric extracts.[6]

(b) *Heart and brain.* Tissues were homogenized twice with 10% TCA and centrifuged. Catecholamines of the combined supernatants were adsorbed on to acid-washed alumina (Woelm, Akt. Stufe I) at pH 8·4, washed with deionized and twice glass-distilled water and then eluted with 0·25 N HCl. All reagents contained EDTA. The eluates were centrifuged during 10 min at 30,000 *g* and 0° in a Servall centrifuge,[7] and stored at −18° until estimation was performed. Storage of eluates never exceeded 24 hr. The recovery of noradrenaline added to tissue homogenates averaged 89 per cent (84–102 per cent). No corrections for incomplete recovery have been made.

Estimation of catecholamines

This was carried out essentially by the method of von Euler and Lishajko[8] except that 10 N NaOH was used rather than 5 N NaOH.[7] Fluorescence of the trihydroxyindoles was measured in a Farrand photofluorometer with the filter combinations used by Cohen and Goldenberg.[9] Each extract was estimated by triplicate analysis. Internal standards of noradrenaline and adrenaline were added to three of every four adrenal extracts, and internal standards of noradrenaline to each extract of brain and heart.

Estimation of enzyme activities

Monoamine oxidase (MAO). Mitochondrial suspensions. The organs of five animals were washed, pooled and homogenized in 0·25 M sucrose. Fractionation of tissues

was performed essentially according to the method of Schneider.[10] The particulate fractions were suspended in M/15 phosphate buffer, pH 7·3, for enzymic assay. For the experiments described here, the mitochrondrial fractions have been diluted so that 1·5 ml of suspension was equivalent to 0·2 g liver, 0·5 g brain, and 0·3 g heart, respectively.

MAO activity was measured by conventional manometric techniques for determination of oxygen uptake. Microdiffusion analysis has been carried out concomitantly for determination of ammonia production by brain and liver mitochrondria. From the fifteenth day of treatment, ammonia production was also estimated in myocardial mitochrondria.

Oxygen uptake was estimated at 37° in Warburg apparatus. Air was used as gas phase. The incubation mixture contained mitochrondrial suspension and substrate in a final vol. of 2 ml. Cyanide and semicarbazide were not added to block secondary oxidations.[11] Tyramine and 5-hydroxytryptamine (Fluka) were used as substrates in a final concentration of 0·01 M. The inner well contained 50% KOH. The results are expressed as μl O_2/g tissue for an incubation period of 2 hr after tipping the substrate. Blank values were carried out by omitting the substrate from the incubation medium. All values for each substrate are means of duplicate analysis. There was a good correlation between single values of duplicate analysis. In more than 110 estimations carried out as duplicates during this experiment, only four showed variations exceeding 5 per cent. These four cases concerned myocardial mitochrondria. Ammonia released during the incubation period was measured in Conway[12] units using 1 ml of the incubation mixture and a solution of K_2CO_3 for alkalinizing the enzyme reaction mixture. The inner well of the units contained boric acid—indicator solution. Titration was carried out with 0·02 N or 0·05 N H_2SO_4. In this series of estimations, tyramine was the only substrate used. The results are expressed as μl NH_3/g tissue, produced during an incubation period of 2 hr. The determinations have been made in triplicate analysis (duplicate for the blank values) and correlation between multiplicates was good. Accuracy was found to be slightly less than with the manometric procedure, although NH_3 liberated from known amounts of NH_4Cl and determined throughout the experiment was recovered almost quantitatively. From a theoretical weight of 85 μg NH_3 the mean recovery was 84·61 $\pm$ 0·3 μg (No. = 21) when 0·05 N H_2SO_4 was used for titration. It reached 84·92 $\pm$ 0·12 (No. = 19) with 0·02 N H_2SO_4.

Catechol O-*methyltransferase* (COMT) *activity*. The method of Axelrod *et al.*[13] was used with some modifications. The tissues were homogenized in tris buffer (liver 1:4; brain and heart 1:3). DL-H^3-noradrenaline (New England Nuclear Corporation) or DL-C^{14}-noradrenaline (Radiochemical Centre, Amersham, England) were used as substrate. β-H^3-noradrenaline (5·8 c/mM) and β-C^{14}-noradrenaline (8·1 mc/mM) were diluted with non-radioactive noradrenaline or with water to give solutions containing 260 mμC (H^3), respectively 22·7 mμC (C^{14}) and 2·8 mμ M noradrenaline in 20 μl. These dilutions contained 2% sodium metabisulphite. The amount of radioactive noradrenaline was determined after incubation for 20 min at 37°. For each experiment organs of two rats were pooled. Triplicate and duplicate analyses were performed for normal and blank values, respectively, the latter being obtained by omitting adenosyl-methionine from the incubation mixture. Adenosyl-methionine was obtained from rabbit liver.[14] All samples were counted in a Packard-Tri-Carb liquid scintillation spectrophotometer

after adding 10 ml of the new scintillator BBOT* and together with H^3- or C^{14} standards. The accuracy of the method averaged ± 11 per cent.

RESULTS

I. *Effect of pyrogallol on the catecholamine content of the adrenals, brain and heart*

The results of catecholamine determinations in the adrenals are shown in Fig. 1A. The amounts of noradrenaline and adrenaline of control rats are fairly variable.

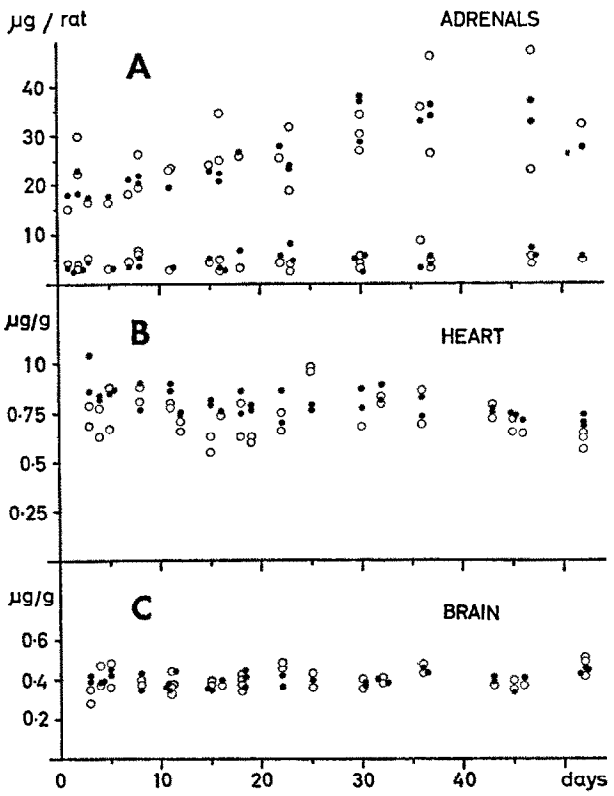

FIG. 1. Effect of daily pyrogallol administration on the catecholamine content of rat organs:

A. ▲ Adrenaline content in the adrenals of saline-treated rats; △ adrenaline content in the adrenals of pyrogallol-treated rats; ● noradrenaline content in the adrenals of saline-treated rats; ○ noradrenaline content in the adrenals of pyrogallol-treated rats.

B. ● Catecholamine content in the heart of saline-treated rats; ○ catecholamine content in the heart of pyrogallol-treated rats. In the heart the concentration of adrenaline never exceeded 4 per cent of that of noradrenaline. Thus the catecholamine content is expressed in terms of noradrenaline.

C. ● Noradrenaline content in the brain of saline-treated rats; ○ noradrenaline content in the brain of pyrogallol-treated rats. The organs of two or three rats were pooled for each determination.

These variations were even more pronounced in the pyrogallol-treated rats, particularly for the adrenaline content. During the first 2 weeks, the mean adrenaline concentration was greater in the pyrogallol-treated rats than in the controls, but the difference shown was not significant. An increase of the adrenaline content in the

* Scintillator CIBA.

adrenals and sometimes a relative decrease of the noradrenaline content have been observed during ontogenesis.[15–19] Although the rats used in this experiment were nearly adult (140–180 g) at the beginning of the treatment, it can be seen (Fig. 1A) that the adrenaline concentration still increased at least for 30–40 days.

Because of variations in the mode of reporting catecholamine content of adrenals, it is important to know that this increase was also noticeable—but only for about 20 days—when the results were expressed in terms of μg/g fresh adrenal, but it could not be demonstrated when the results were expressed in terms of μg/kg rat. These considerations can be made for both pyrogallol-treated and control rats.

As seen in Fig. 1C, the noradrenaline stores of the rat brain were not affected by chronic administration of pyrogallol, at least when determined 22–24 hr after the last injection. In contrast to the results obtained from the adrenals, the noradrenaline concentration in the brain of pyrogallol-treated rats was not more variable than that of control brains. There was also no modification of the noradrenaline content of the brain during the whole observation time, either in the pyrogallol or in the saline-treated rats. Moreover, the mean concentration of the single estimations corresponds to the mean results obtained from normal rats in other experiments. Adrenaline usually has been found in trace amounts, but in some experiments no adrenaline could be detected.

The results of catecholamine estimations in the heart are shown in Fig. 1B. Since the concentrations of adrenaline never exceeded 4 per cent of that of noradrenaline, the total cardiac catecholamines are expressed in terms of noradrenaline. The noradrenaline content of control hearts was quite high at the beginning of the treatment period and it dropped slightly during the experiment. However, in the hearts of pyrogallol-treated rats, this drop was not noticeable during the first weeks of the treatment, but it was even more pronounced than in controls during the last weeks. Another comparison between both periods must be made. In the control hearts the changes of the noradrenaline content was rather regular during the whole duration of the experiment, whereas the noradrenaline concentration in the hearts of treated animals was variable during the first weeks and decreased then at a fairly constant rate. The physiological variations, as estimated from single determinations, carried out at the same time were usually not greater than those encountered in saline-treated animals. Since the fluctuations of the noradrenaline stores in the heart are relatively high in both groups, a valid statistical analysis would need more extracts in each individual case. Indeed, it would be incorrect to pool the results of each group for statistical evaluation, but it can be said that certainly no marked modification occurred in the cardiac noradrenaline stores during pyrogallol treatment. Nevertheless, the chronic administration of pyrogallol might produce a slight catecholamine depletion in this organ in contrast to the observations made in the brain and in the adrenals.

II. *Effect of pyrogallol on monoamine oxidase activity of liver, heart and brain*

The results of MAO activity of liver, heart and brain during long term administration of pyrogallol are given in Fig. 2. For the three organs and for both NaCl and pyrogallol-treated rats, a good correlation has been found between oxygen uptake and ammonia evolution.

The activity of liver monoamine oxidase is shown in Fig. 2A. Pyrogallol administration had a biphasic action. Indeed, a slight increase of enzymic activity has been

noted between the eleventh and the twenty-second day of treatment, while no change occurred before. After the third week there was a fairly constant drop in monoamine oxidase activity.

In the heart, monoamine oxidase activity (Fig. 2B) of controls increased during the period of treatment. This is in agreement with the results of other authors[20, 21] who

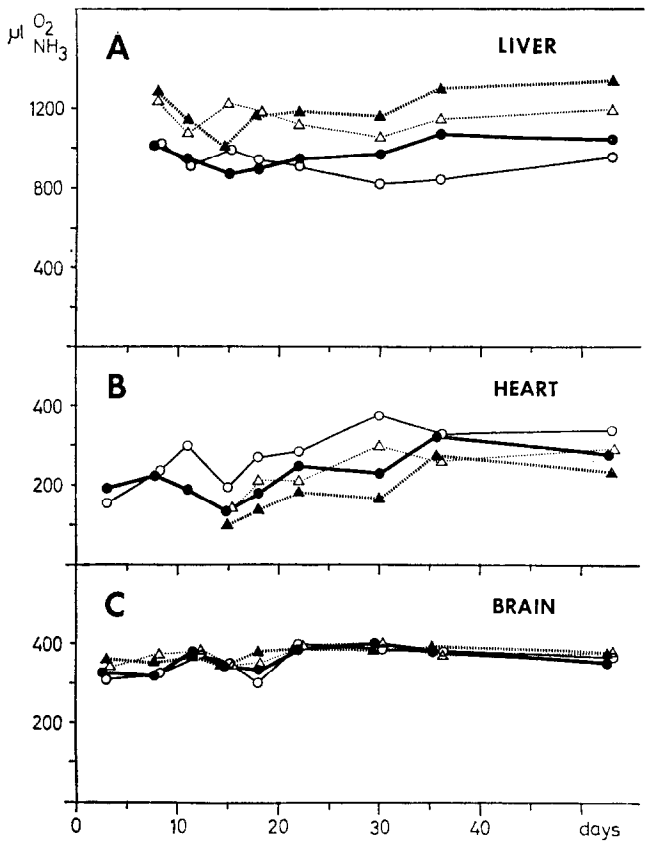

FIG. 2. Effect of daily pyrogallol administration on monoamine oxidase activity of rat organs:
A, B and C. µl of O_2 taken up (solid lines) and µl of NH_3 liberated (dotted lines) during an incubation period of 2 hr, and calculated for 1 g of organ.
Black symbols values in organs of saline-treated rats; Open symbols values in organs of pyrogallol-treated rats.
These experiments have been performed with tyramine as substrate and the results have been compared with those obtained by using 5-hydroxytryptamine as substrate (see Table 1).
The organs of 5 rats were pooled for each determination.

have found an increased monoamine oxidase activity in the heart of old rats. Cardiac monoamine oxidase activity of pyrogallol-treated rats increased slightly faster than that of controls at the beginning of the treatment and during a further 10 days. Afterwards the increase of both series was of the same order of magnitude. From the eighth day, monoamine oxidase activity was greater in the pyrogallol group.

The activity of brain monoamine oxidase of control rats remained fairly constant during the treatment period, and pyrogallol administration did not affect this activity (Fig. 2C).

It must be pointed out that the ratio oxygen consumed with 5-hydroxytryptamine as substrate/oxygen consumed with tyramine as substrate varies from organ to organ in the rat (Table 1). In liver, heart and brain this ratio diminished with time. After an incubation period of 2 hr, oxygen uptake was equal for 5-hydroxytryptamine and tyramine in the brain. It was greater for 5-hydroxytryptamine in the heart and greater for tyramine in the liver.

TABLE 1. COMPARISON OF MONOAMINE OXIDASE ACTIVITIES OF RAT ORGANS, USING 5-HYDROXYTRYPTAMINE OR TYRAMINE AS SUBSTRATES

Treatment	No.	Organ	Incubation time (min)		
			30	60	120
NaCl 0·9%	11	Liver	1·034 ± 0·016	0·930 ± 0·012	0·859 ± 0·009
	10	Heart	1·591 ± 0·042	1·370 ± 0·017	1·217 ± 0·013
	9	Brain	1·337 ± 0·017	1·134 ± 0·015	0·991 ± 0·009
Pyrogallol	8	Liver	1·048 ± 0·024	0·935 ± 0·020	0·858 ± 0·014
50 mg/kg per day	9	Heart	1·584 ± 0·026	1·356 ± 0·024	1·202 ± 0·019
	9	Brain	1·414 ± 0·025	1·164 ± 0·022	1·010 ± 0·007

Numbers indicate the ratio (mean ± S.E.): μl O_2 consumed when 5 hydroxytryptamine was used as substrate/μl O_2 consumed when tyramine was used as substrate.
Since the different ratios were very stable during the whole experiment, the single determinations have been pooled for this table.
The organs of five animals were pooled for each determination.

These results present further evidence that various tissues contain either different single monoamine oxidase or different proportions of several monoamine oxidases, as suggested by other multiple substrate and/or inhibitor studies.[22–26]

Pyrogallol had no effect on these different ratios, in contrast to several monoamine oxidase inhibitors (Maître, manuscript in preparation).

III. *Effect of pyrogallol on catechol-O-methyltransferase activity*

Two kinds of measurements have been performed in order to evaluate the acute responsiveness to the inhibitor as well as its chronic effects.

—COMT activity was measured 22–24 hr after the last injection in both groups (Fig. 3A).

—COMT activity was measured 30 min after the last injection of pyrogallol. Rats treated daily with NaCl received a single injection of pyrogallol 30 min before death and were used as controls (Fig. 3B).

Enzyme activity was determined in the liver throughout the experiment. In addition, it was determined also in brain and heart during the first 2 weeks.

(a) COMT *activity 30 min after the last pyrogallol injection*

When liver COMT was measured 30 min after the administration of pyrogallol into rats of both groups, a strong inhibition was noted in each case. This was usually less in the rats which had received daily injections of pyrogallol than in the controls. The results which could be analysed statistically showed no significant difference between both groups during the first 2 weeks. They are in agreement with another series of

experiments performed during 52 days where one extract/day of observation has been analysed. Thirteen determinations carried out during this time gave no further information.

COMT was also found to be inhibited in heart and brain. Nevertheless, the inhibition was less pronounced than in the liver. In the brain significant inhibition ($P < 0{\cdot}05$) was found for control and treated rats. In the last group, however, the inhibition was somewhat smaller.

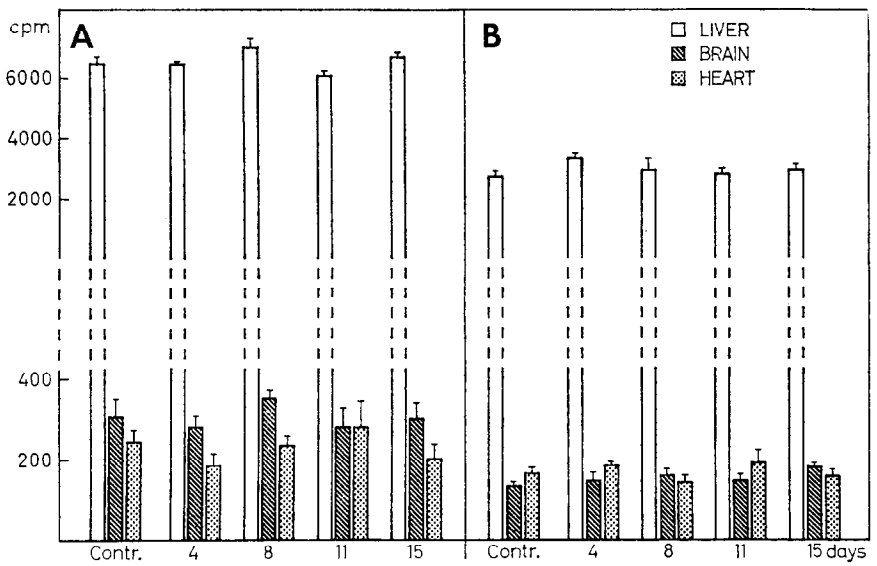

FIG. 3. Effect of daily pyrogallol administration on catechol-O-methyltransferase activity of rat organs:

A. COMT activities 24 hr following the last saline (controls) resp. pyrogallol injection;

B. COMT activities 30 min following the last pyrogallol injection. Control rats were treated daily with saline and received a single injection of pyrogallol 30 min before removal of the organs.

The experiments have been performed using DL-C^{14}-noradrenaline as substrate (see Methods). The organs of two rats were pooled for each determination. Columns represent mean value (± S.E.) of three to five determinations.

In contrast to experiments on liver and brain, the inhibition produced by pyrogallol on cardiac COMT was less pronounced. Although it was demonstrable in each experiment, it was significant ($P < 0{\cdot}05$) only after 5 days, in the treated group. All other P values exceeded 0·05. It has been already well established that heart tissue contains only little COMT activity.[27–29] There is, however, very little information about the inhibiting properties of pyrogallol on cardiac COMT. The results presented here show that COMT of rat heart is relatively resistant to pyrogallol action.

(b) COMT *activity* 22–24 *hr after the last pyrogallol injection*

Twenty-two to twenty-four hours after the last injection of pyrogallol or NaCl, respectively, COMT activity was essentially the same in control and treated rats. These

results are in agreement with those of the 52 days experiment. In this series, seven determinations have been performed during the first 15 days from the beginning of treatment. During this time COMT activity from treated rats showed little and irregular variations when compared to that of controls. It ranged between 94 and 135 per cent after 3 and 8 days, respectively, with a mean value of 108·5 per cent. From the 18th day until the 52nd day, no greater modification was noted. COMT activity of the pyrogallol-treated group was slightly higher and averaged 107·5 per cent (range: 102 to 112 per cent).

Twenty-two to twenty-four hourr after the last injection of pyrogallol, COMT activities of heart and brain had returned to control values, as in the liver, but standard errors of the mean were greater than in the liver.

DISCUSSION

The lack of activity of pyrogallol on brain monoamine oxidase activity contrasts with the changes in monoamine oxidase activities shown in heart and liver. Thus a biphasic effect of pyrogallol on hepatic monoamine oxidase has been noted. It was characterized initially by a slight increase above control values, and from the third week by a more pronounced decrease.

In contrast to the decrease in hepatic monoamine oxidase activity produced by long term administration of pyrogallol, a small increase in cardiac monoamine oxidase activity has been noted. The results of the determinations of monoamine oxidase activity in these organs substantiate other findings from which it should be considered that the metabolic pathways of adrenaline[30] and noradrenaline[31] probably vary considerably from organ to organ within the same species.

Pyrogallol is, above all, an inhibitor of COMT. Thus the results of its effect on COMT activities on various organs was considered of primary importance among the experiments described here. Since Nukada *et al.*[5] had found considerable changes in the excretion of metanephrine as well as of vanillylmandelic acid during the first 2 or 3 weeks of pyrogallol treatment, it was important to know if this effect could be correlated directly with a chronic effect of pyrogallol or with a modification of the acute responsiveness of tissue COMT to this inhibitor. In our experiments, COMT activity did not show strong changes on pyrogallol treatment. In the most cases, it was increased slightly 24 hr after the last pyrogallol injection but this increase never differed significantly from the COMT activity of control rats (P always exceeded 0·05). A similar observation could be made 30 min after pyrogallol injection; this indicates that the responsiveness to pyrogallol remained practically unchanged throughout the experiment. The biphasic changes reported by Nukada *et al.*[5] have not been observed in the present experiment neither as an acute effect nor as a chronic effect of pyrogallol administration. Furthermore a close parallelity was not found between the changes of monoamine oxidase and COMT activities. These discrepancies cannot be explained at present.

The interesting assertion that a relationship exists between the change in the rate of excretion of O-methylated derivatives in urine of animals treated with pyrogallol, and a change in the activities of liver monoamine oxidase and COMT could therefore not be corroborated. But it must be said that this conclusion has been established from experiments performed using rabbits for urine analysis and rats for liver analysis.

There is no information establishing that both species show the same metabolic pathways of noradrenaline and adrenaline under the experimental conditions used.

It is noteworthy that the cerebral and cardiac COMT are not inhibited during the acute phase of pyrogallol action more than the hepatic COMT although the endogenous activities of this enzyme are much smaller in the brain than they are in the liver. In fact, the liver was found to be twenty-eight to thirty-five times as active as brain and heart, respectively. It must therefore be assumed that the hepatic COMT is much more sensitive to pyrogallol treatment than are the cerebral and especially the cardiac COMT. Indeed, there is no indication that selective time courses of the pyrogallol action in different organs might explain such differences (Crout[4]). There are two modes of action which must be considered in evaluating this preferential effect: first, the ability of these tissues to take up and retain the inhibitor as well as its accessability to the COMT within the cell. Second, the fact that several kinds of COMT might exist showing different sensitivities to pyrogallol and/or different affinities to other substrates than noradrenaline. To our knowledge, there is no information from previous studies about whether one of these mechanisms could correspond to these individual differences.

The effects of long-term administration of pyrogallol on "endogenous catecholamine levels" have been estimated here from the concentration of these amines in three organs: adrenals, heart and brain. These tissues are important storage sites for catecholamines. Nevertheless, it must be kept in mind that catecholamines are also present in many other tissues which might play a role under the experimental conditions used for these experiments. Indeed, Axelrod and his co-workers presented sufficient evidence that the principal route of metabolism of circulating catecholamines involves meta-O-methylation by COMT. Since pyrogallol is an effective inhibitor of this enzyme it could be assumed that plasma levels of catecholamines are possibly more markedly affected by pyrogallol treatment than the levels of other tissues. However, the results from several studies performed with radioactive catecholamines[31–33] show that such an increase in plasma catecholamine levels is not always detectable and, if present at all, it would be of very short duration. In another type of experiment, Crout *et al.*[31] injected a large dose (20 mg/kg) of *l*-noradrenaline. They found a much higher concentration (ten to thirty times) of noradrenaline in the plasma of rats which had received pyrogallol than in the plasma of control animals. The rate of disappearance of plasma noradrenaline was of course considerably slower in the pyrogallol pretreated rats, but noradrenaline concentration showed a strong drop as soon as 35–50 min after its administration, although a relatively high dose of pyrogallol (200 mg/kg) was used. These results support the view that the rate of disappearance of high concentration of noradrenaline from the plasma is regulated predominantly by COMT activity. It appears, therefore, that the changes in plasma catecholamine levels have had a minor rôle in the experiments described here.

The noradrenaline content of the heart usually was slightly smaller in the treated animals than in the controls. The mechanism of this decrease cannot be assessed by this study. It could be due either to the overactivity of cardiac monoamine oxidase, or to a decrease in the noradrenaline synthesis in the heart, or to a diminished binding capacity for noradrenaline in the cardiac nerve endings. In connection with the later hypothesis it has been observed that pyrogallol elevates the concentration of H^3-noradrenaline in almost all tissues of the cat and particularly in the heart.[33] This

finding supports the view that the decrease observed in our experiments would be a late effect of pyrogallol or that the mechanism of noradrenaline uptake is different in the cat and in the rat. This is likely since opposite effects have been shown to occur in the noradrenaline content of the heart in rats and cats after administration of monoamine oxidase inhibitiors.[34]

It would appear that these experimental results can be reconciled with the concept that pyrogallol represents a poor inhibitor as far as long-term study is concerned. Indeed, the chronic administration of pyrogallol produces only small effects which cannot be attributed to its enzyme inhibiting potency. It never was possible to observe fairly strong changes in the tissue catecholamine content like those which have been observed during the acute anti-COMT-effect of pyrogallol.[35, 36] On the other hand, it would not be possible to inject greater doses in this kind of experiment since the chosen dose (50 mg/kg per day) already produces tissues necrosis at the injection site. Finally, the effects reported here could not substantiate the suggestion that the altered levels of endogenous catecholamines produced by pyrogallol treatment may be regulated by an adaptive change in the activities of COMT and MAO. In this context, these effects can be considered as unspecific reactions which were more important in the cardiac muscle than in the cerebral tissue.

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