## Effect of 3,4-Dimethoxyphenylethylamine Injections Upon Dopamine Metabolism in Rats and Dogs

Substances chemically related to dopamine (3-hydroxytyramine) have been known to possess pharmacological effects. Such is the case for Mescaline[1] and 3,4-dimethoxyphenylethylamine (DIMPEA or D.M.P.E.)[2]. The latter substance can produce in animals an akineto-rigid syndrome akin to the bradykinesia of Parkinson's disease and to the catatonia of Schizophrenia[2,3]. Recently BARBEAU et al.[4] observed a selective increase in urinary dopamine excretion after an injection of DIMPEA to monkeys. In male albino Sprague-Dawley rats, a single injection of DIMPEA caused an increase in the concentration of dopamine in the central grey nuclei of the brain at the time of maximal clinical akinetic effect[5]. This apparent interference of DIMPEA with dopamine metabolism may be of importance since a DIMPEA-like product has been isolated in the urine of parkinsonians[6-8] and since dopamine appears to be implicated in the etiology of bradykinesia in Parkinson's disease[9-11]. The present paper intends to establish on a statistical basis, which was difficult with monkeys[4], the effect of DIMPEA injections upon dopamine metabolism and also to study this effect in other species.

*Experimental.* 24 male albino Sprague-Dawley rats, weighing from 250–400 g (mean: 325 g) were utilized. 2 rats were placed in each metabolic cage according to a table of random numbers. The animals were fed a constant diet and kept in a room at 30 ± 2 °C. All urines were collected for 24 h in bottles containing 1 ml of 6 N hydrochloric acid and stored at 4 °C at a pH always inferior to 3. Determinations were carried out within 72 h of collection. Completeness of the urinary collections was checked by determination of the creatinine content, which in fact did not vary during this experiment (0.63 ± 0.04 mg/ml).

The urines were picked up and the animals fed at the same hour during the whole period. On each urine specimen, determination of dopamine, noradrenalin and adrenalin were carried out according to the method of SOURKES and MURPHY[12]. Homovanillic acid (HVA) was estimated with the procedure of SATO[13]. Control experiments demonstrated that presence of DIMPEA or of its metabolites even in high concentration in the urine did not interfere significantly (less than 1%) with the determination of HVA using the procedure of SATO. The animals were randomized into 3 groups of 5 cages. 'Group A' was given, on the morning of the fifth day, an injection of 100 mg/kg of DIMPEA (i.p.). The same day, 'Group B' received an equivalent volume of physiologic saline while 'Group C' was left intact but manipulated. The experimental protocol called for successive 24 h collections during 4 control days and 7 days post-injection.

As a control experiment (see discussion) a female mongrel dog weighing 21 kg was injected under Pentobarbital anaesthesia with 10 mg/kg of DIMPEA i.p. on 2 occasions (experiments 2 and 4) or with an equivalent volume of physiologic saline (experiments 1 and 3). These experiments were carried out at weekly intervals. Urine was collected through a catheter at $^1/_2$ h intervals before and after the injection and the same determinations were carried out as outlined above.

*Results.* It can be seen in Figure 1 and in the Table that the injection of DIMPEA (100 mg/kg) to rats failed to demonstrate the selective increase in dopamine urinary excretion that had been observed previously in monkeys[4]. There was a tendency for adrenalin excretion to be increased but this did not reach statistical significance at the 2% level (in fact for Group A, $p = 0.05$ between DIMPEA injection day and the control period but not significant when compared for the same day with other groups). However, the excretion of homovanillic acid (HVA), the main metabolite of dopamine, was significantly increased ($p < 0.01$) in the 24 h following the injection (Figure 2).

A discrepancy of this nature could be due to the fact that in rats the turnover rate of catecholamines is such


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Statistical analysis

| Substance measured | Injection day-vs-first 4 days | | Injection day | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Group A | | Group A/Group B | | Group A/Group C | | Group B/Group C | |
| | *t* | *p* | *t* | *p* | *t* | *p* | *t* | *p* |
| Dopamine | 0.80 | N.S. | 1.67 | N.S. | 0.52 | N.S. | 1.33 | N.S. |
| Noradrenalin | 1.24 | N.S. | 0.68 | N.S. | 0.71 | N.S. | 0.25 | N.S. |
| Adrenalin | 2.09 | N.S. | 2.00 | N.S. | 1.89 | N.S. | 0.20 | N.S. |
| HVA | 3.34 | $< 0.01$ | 3.48 | $< 0.01$ | 3.47 | $< 0.01$ | 1.12 | N.S. |

All other relationships between groups are non-significant (N.S.) at 2% level of probability.

that a temporary increase in the excretion of dopamine would be lost in the 24 h collection. Because of the small urinary volume of rats it was impossible to check this hypothesis in the same species. In 2 experiments on a female mongrel dog, however, it was seen (Figure 3) that an injection of DIMPEA (10 mg/kg, i.p.) produced a significant increase in the excretion of dopamine ($p < 0.01$ over paired control experiment) within the first 30 min post-injection, followed 30–50 min later by a significant elevation of HVA excretion ($p < 0.02$ over paired control experiment).

*Discussion.* Taken together with the previously reported findings in monkey urine[4] and in rat brain[5], the present results appear to confirm the hypothesis that DIMPEA modifies the metabolism of dopamine, either through interference with the re-uptake mechanism or through specific receptor blockade. This is manifested in dog by an almost immediate overflow of dopamine and in rat brain, at the time of maximum akinetic effect (from 10–30 min post-injection), by a significant increase in dopamine concentration within the central grey nuclei and not the rest of the brain[5]. This interference with dopamine (pre or postsynaptic?) probably stimulates in dopaminergic fibres a compensatory synthesis of dopamine resulting in a marked increase in HVA and possibly also, but later, in noradrenalin and adrenalin. A similar feed-back mechanism has been proposed as a mode of action for various neuroleptics, notably chlorpromazine and haloperidol[14–17]. Da Prada and Pletscher[18] explain the increase of cerebral HVA level after chlorpromazine by an accelerated turnover of dopamine resulting from specific dopaminergic receptor blockade in the extrapyramidal centres. It is thus of interest that DIMPEA, like the above mentioned neuroleptics, can also produce extrapyramidal complications (i.e. akinesia) when injected to animals[2,3]. A reserpine-like effect of DIMPEA is improbable in view of the fact that noradrenalin and 5-HIAA excretion are not modified at the same time[4]. Experiments are underway in our laboratory to determine the effect of chronic injections of DIMPEA on dopamine

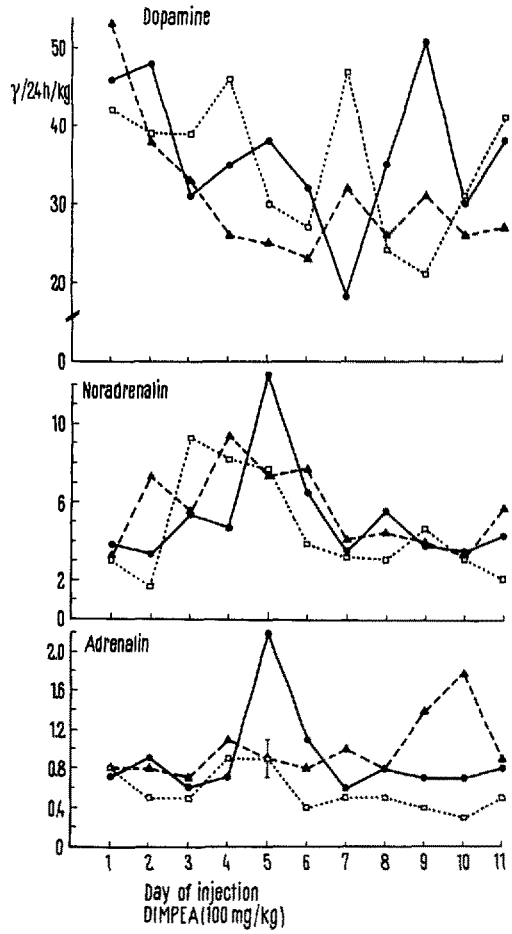


Fig. 1

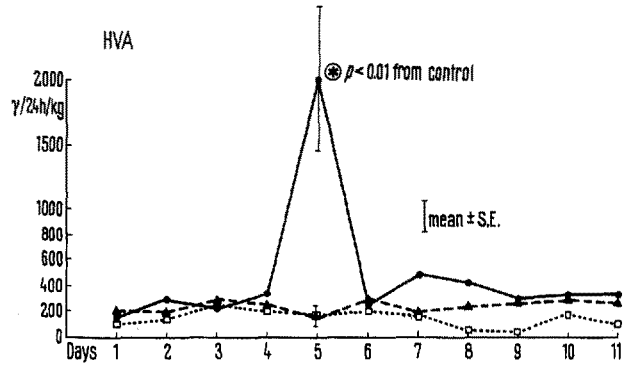


Fig. 2

Figs. 1. and 2. Every point on this graph represents the mean of 5 experiments. Standard error is indicated only when the difference is significant at the 2% level. ●—● DIMPEA, ▲----▲ NaCl, □···· □ control. Day 5 = day of injection.

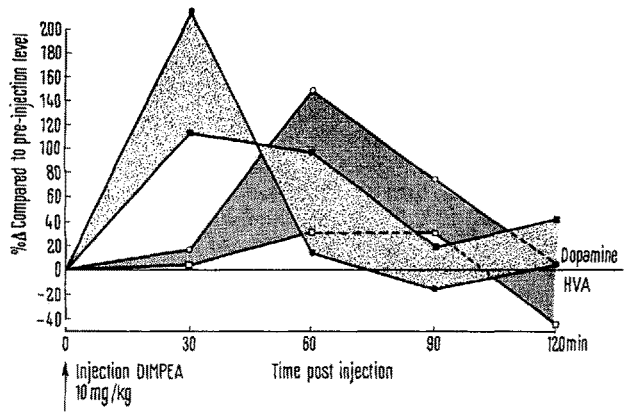


Fig. 3. Effect of DIMPEA upon dopamine metabolism (dog). This graph represents the % variation over a base level established as point zero. This base level was obtained by pooling the results of 3 consecutive 30 min periods pre-injection. For each of the 2 experiments, the results are expressed after correction for the paired control experiment (physiologic saline injection). ●——● D.A., o——o HVA, □ first experiment, ○ second experiment.

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metabolism, and to study the pharmacological mechanism of action of DIMPEA at the synaptic terminals of dopaminergic fibres[19].

*Résume.* L'effet d'une injection de 100 mg/kg de 3,4-diméthoxyphényléthylamine (DIMPEA) sur l'excrétion urinaire des catécholamines a été étudié chez le rat en cage métabolique. Il est montré que le DIMPEA produit une augmentation significative de l'excrétion urinaire d'acide homovanillique (HVA), le principal métabolite de la dopamine. Le mécanisme d'action plus précis a été étudié chez le chien et il est proposé que le DIMPEA produit une accélération du «turnover» de la dopamine par une augmentation de synthèse de l'amine, elle-même consécutive à un bloc au niveau des terminaisons dopaminergiques.

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## An Effect of Diet on Homovanillic Acid Excretion

Recently, VON STUDNITZ and NYMAN[1] reported that a chromatographic spot corresponding to dimethoxyphenethylamine (DMPEA) disappeared from the urine of 9 subjects when they were fed a glucose diet flavored with citric acid. VON STUDNITZ interpreted these data to signify that DMPEA was present in the diet although he did not analyze food material for the presence of this compound. While exclusion diets have frequently been used to determine whether a compound is derived from exogenous sources, it is questionable whether the disappearance of a compound from urine during this kind of regimen is evidence that the compound is of dietary origin. For example, GOODMAN[2] has shown that the withdrawal of food for 24 h results in a sharp decrease in the excretion of vanillylmandelic acid (VMA) in human subjects, although this compound is known to be a metabolic product of noradrenaline.

In order to investigate some of the complex relationships involved in dietary studies, a study of the effect of a glucose diet on a known metabolic product was undertaken. It has previously been shown that homovanillic acid (HVA) is the major metabolite of dopamine. In rats about 60% of administered dopamine $C^{14}$ is converted to HVA[3]. VON STUDNITZ et al.[4] studied the effects of a glucose diet in humans on the excretion of this acid as well as a number of other acids. He found that HVA was excreted during the glucose diet, but he did not report whether there was a change in its concentration. In the present study rats were studied rather than humans inasmuch as complete dietary control is much more readily obtained in laboratory animals.

*Procedure and Results.* Two 250 g Holzman rats were placed together in each of 2 metabolic cages and offered an ad libitum diet of Wayne Lab Blox. The rats were permitted to accommodate to the laboratory and the cage for several days before the experiment was started. Water was not restricted. At the end of this time, food was removed, the cages were cleaned and a urine sample was collected for 24 h, from each group of rats, in a vessel containing 5 $cm^3$ of 0.1 *N* HCl. At the end of the 24 h urine collection the rats were offered a diet consisting of 5% glucose solution in water. After 24 h of this diet urine collection was started and continued for the next 24 h. The entire 24 h sample was assayed by the method of ARMSTRONG et al.[5], except that the extract was chromatographed on a 1 dimensional strip, ascending. The area corresponding to HVA was then eluted and chromatographed on a second 1 dimensional strip, also ascending. The solvents were respectively: isopropanol-ammonia-water, 8:1:1, and benzene-isopropanol-water, 2:2:1. The area corresponding to HVA on the strips from the pre-glucose urines was compared with those obtained from urines collected after glucose diet. All strips showed a strong area corresponding to HVA before glucose diet. Post glucose strips did not contain detectable amounts of HVA, however.

In order to preclude the possibility that HVA was present in the Wayne rat food used in this study, 6 pellets of rat food were subjected to analysis for HVA. This amount is in excess of the amount fed to each group of rats during the 24 h period. The pellets were mashed in 0.1 *N* NaOH, until a fine slurry was produced, and then filtered. The solution was extracted and chromatographed as described for the urine samples[5]. No spot corresponding to HVA was detectable.

6 additional pellets were also treated by a procedure devised for the extraction of phenolic acids from foodstuff[6]. The extract obtained by this procedure was chromatographed in 2 consecutive 1 dimensional systems, as above. After the second chromatographic separation, the area corresponding to HVA was eluted with methanol, rechromatographed on 1 dimensional thin layer plates coated with silica gel and developed in butanol-ethyl acetate-ammonia, 3:1:1, ascending. Again no spot corresponding to HVA could be detected in the rat food.

A third group of rats was also subjected to a glucose diet as described for the other 2 groups, and urine was collected and processed as detailed for the other urine samples. The area corresponding to HVA was eluted after

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