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Acid metabolites of monoamines in avian brain; effects of probenecid and reserpine

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Summary

1. The concentration of the dopamine (DA) metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the anterior part of the nucleus basalis of pigeon brain was found to be $0{\cdot}17 \pm 0{\cdot}01$ μg/g, which is about one-fifth of the concentration of homovanillic acid (HVA) in this region. In the chicken, the concentration of HVA in the (entire) nucleus basalis was $0{\cdot}06 \pm 0{\cdot}006$ μg/g, lower than in any other species examined, and giving a ratio of DA to HVA of about 50. The concentration of DOPAC in the 8 day old chick was $0{\cdot}053 \pm 0{\cdot}002$ μg/g.

2. Probenecid, 200 mg/kg intramuscularly, doubled the content of DOPAC in the nucleus basalis of the pigeon and increased the concentration of HVA in both the pigeon and the chicken by a factor of 4 to 5. These findings demonstrate the existence, in avian brain, of an active transport mechanism for the removal of acidic substances and explain the low concentrations of the acids found in bird brain.

3. A method is described for the estimation of 5-hydroxytryptamine (5-HT) and 5-hydroxyindolylacetic acid (5-HIAA) in the same tissue sample. Probenecid caused an increase in the 5-HIAA content but produced no change in the 5-HT content of the nucleus basalis of pigeon brain.

4. Reserpine caused a fall in the content of acidic DA metabolites in the nucleus basalis of the pigeon. The effect was more pronounced after raising the concentration of these acids with probenecid.

5. Treatment of pigeons with pargyline (100 mg/kg 17 hr before decapitation) did not significantly increase the DA content of the nucleus basalis, but it prevented to some extent the loss in DA caused by reserpine.

6. Pigeons and chickens were sedated by probenecid. The deepest sedation occurred at about the same time as the greatest increase in the acidic amine metabolites in the brain.

7. Intracisternal injection of HVA in the pigeon and intravenous injection of large amounts of HVA, DOPAC, 5-HIAA or 3,4-dimethoxyphenylacetic acid into newly hatched chicks did not produce any sedation or other effects on behaviour. In contrast, injection of sodium γ-hydroxybutyrate caused paralysis followed by prostration and eye closure.

8. Estimation of the concentration of HVA in the brain of the young chick after intravenous injection of the acid (100 mg/kg) showed that the concentra-

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tion was of the same order of magnitude as it is in animals given probenecid ; this suggests that the sedation which follows probenecid is not related to the accumulation of acidic amine metabolites.

Introduction

An active transport mechanism for removal of acidic metabolites of 5-hydroxytryptamine (5-HT) and dopamine (DA) has been found in the brain of the rat and the mouse (Neff, Tozer & Brodie, 1964 ; Sharman, 1966, 1967, 1969 ; Werdinius, 1966, 1967b, 1968). In the brain of these rodents the concentrations of the amines are much higher than the concentrations of the acid metabolites, and there is a large rise in the acid metabolite concentration after the administration of probenecid, a substance known to inhibit the transport of acidic substances in the kidney. No such rise is obtained in animals, for example, rabbits, in which the brain concentration of the acid metabolites is high (Werdinius, 1967b).

Juorio & Vogt (1967) found that in the pigeon brain the ratio of DA to its main acidic metabolite 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid ; HVA) is about 4 to 1, which is intermediate between that of the two groups of mammals. In order to see whether this ratio indicated the presence of a transport mechanism for organic acids in the bird brain, we have studied the effect of probenecid on the concentration of HVA and of another acidic metabolite of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) in pigeon and chick. The effect of probenecid on pigeon brain 5-hydroxyindol-3-yl acetic acid (5-HIAA), the concentration of which is about half that of the corresponding amine 5-hydroxytryptamine (5-HT), was also examined. Furthermore the effects on behaviour of injecting large doses of acid metabolites were compared with those seen after administration of probenecid.

Experiments were also carried out to see the combined effect of reserpine and probenecid on these acid metabolites, because in pigeon brain reserpine does not increase the concentration of HVA (Juorio & Vogt, 1967), whereas in mammals reserpine treatment does (Andén, Róos & Werdinius, 1964).

Methods

Adult pigeons of either sex weighing 300–500 g were used for most of the experiments. Some experiments were carried out on chicks aged between 1 day and 2 weeks and on adult chickens weighing about 1 kg. During the experiments the birds were allowed food and drink *ad libitum*. After decapitation the brain was rapidly removed and dissected according to Juorio & Vogt (1967). The part of brain analysed was the anterior part of the nucleus basalis ; it is referred to as " nucleus basalis " in the text. When, as done in the chick, the whole of the nucleus basalis was analysed, this is indicated in the text. Injections were made into the breast muscle, the wing vein or the cisterna magna of pigeons, and into breast muscle, wing vein or jugular vein of chickens.

Drugs

The drugs used were: probenecid (Benemid, Merck Sharp & Dohme Ltd.) ; this was dissolved in a minimum volume of 0·1 N NaOH, an approximately 5% solution made by adding saline, and the pH adjusted to 7–8 with 0·1 N HCl ; reserpine (Serpasil, Ciba) ; a 0·1% solution was prepared in 10% ascorbic acid and diluted

further with saline; pargyline (Abbott Laboratories, dissolved in saline); D,L-3,4-dihydroxyphenylalanine (DOPA) (Koch-Light Laboratories Ltd., dissolved in dilute HCl); homovanillic acid (Calbiochem); 3,4-dihydroxyphenylacetic acid (Koch-Light Laboratories Ltd.), recrystallized once from benzene; 5-hydroxyindolylacetic acid (Koch-Light Laboratories Ltd.); 3,4-dimethoxyphenylacetic acid (Koch-Light Laboratories Ltd.); sodium γ-hydroxybutyrate prepared from γ-butyrolactone (Koch-Light Laboratories Ltd.).

For injections of HVA into the cisterna magna an isotonic solution was prepared in water, its pH adjusted to 7 with $NaHCO_3$, and bromophenol blue was added to indicate the distribution of the injected fluid inside the skull. The various organic acids injected intravenously were prepared in the same way but without the addition of dye.

Chemical estimations

HVA and DOPAC

Homovanillic acid and 3,4-dihydroxyphenylacetic acid were estimated as described by Murphy, Robinson & Sharman (1969). The tissue samples were homogenized in ice-cold 0·1 N HCl and kept at 4° C throughout the extraction procedure. Tissue proteins were precipitated with perchloric acid, and solid KCl was used to remove the perchlorate as insoluble $KClO_4$. After centrifugation the acids were extracted from the supernatant into *n*-butyl acetate. Tris (trihydroxymethyl-methylamine) solution was used to extract HVA and 1,2-diaminoethane to extract DOPAC from the *n*-butyl acetate.

The acids were estimated fluorimetrically in an Aminco-Bowman spectrophotofluorimeter. The method of Andén, Roos & Werdinius (1963) was used to estimate HVA. The extracts containing DOPAC in 1,2-diaminoethane were incubated in a water bath at 63° C for 20 min in the dark, they were then rapidly cooled, acidified with 6 N HCl, and 10 min later the samples were neutralized by addition of 1,2-diaminoethane and the resulting fluorescence was measured.

When HVA was estimated in plasma metaphosphoric acid was used to precipitate the proteins instead of perchloric acid (Werdinius, 1967a), and the acid was then extracted into *n*-butyl acetate as described above.

Dopamine

Dopamine was estimated as follows (Bertler, Carlsson, Rosengren & Waldeck, 1958; Laverty & Sharman, 1965): Tissue samples were homogenized in 0·1 N HCl containing ascorbic acid 0·1 mg/ml. Proteins were precipitated with perchloric acid. After centrifugation at 4° C the pH of the supernatant was adjusted to 4 with dilute K_2CO_3 solution and the samples were centrifuged to remove the $KClO_4$. Dopamine in the clear supernatant was adsorbed on to columns of the resin Dowex 50×8 which had been previously treated with 2 N HCl and 1 M Na acetate buffer and washed with distilled water. After the supernatant had run through, the columns were washed with 0·4 N HCl and dopamine was eluted with 2 N HCl. The dopamine in the eluates was acetylated with acetic anhydride and $NaHCO_3$ and condensed with 1,2-diaminoethane. After extraction into isobutanol the fluorescence derived from dopamine was estimated in a Locarte fluorimeter (Laverty & Sharman, 1965).

5-HT and 5-HIAA

5-Hydroxytryptamine and 5-hydroxyindolylacetic acid were estimated in the same tissue sample by a combination of methods described by Bertler (1961) and Contractor (1966). Tissue samples were homogenized in ice-cold 0·1 N HCl containing ascorbic acid and diluted with an equal amount of water. The proteins were precipitated by bringing the homogenate to 0·4 N with respect to perchloric acid. After centrifugation the clear supernatant was adjusted to pH 4 with KOH using an automatic titration apparatus. The extract was then centrifuged to remove $KClO_4$ and the supernatant passed through a 45 × 5 mm column of Amberlite GC 50 prepared in the sodium form. The effluent of the column was collected and acidified with 0·2 ml. of 2 N HCl and then passed through a 25 × 5 mm column of Sephadex G10, which had been prepared by washing first with 5 ml. 0·2 N ammonia containing 0·1 mg/ml. ascorbic acid and then with 5 ml. of 0·1 N HCl. The Sephadex G10 column was washed with 0·1 N HCl and the 5-HIAA eluted with 2 ml. 0·2 N NH_4OH containing 0·1 mg/ml. ascorbic acid. The Amberlite GC 50 column was washed with water and the 5-HT eluted with 3 ml. 0·2 N HCl. Both eluates were made 3 N with respect to HCl and the fluorescence measured in an Aminco-Bowman spectrophotofluorimeter.

With these methods, recoveries of compounds added to homogenates in amounts approximating those expected in the samples were (means ± S.E.M., number in parentheses): HVA 61·3 ± 2·3% (19), DOPAC 59·4 ± 1·8% (24), DA 78·6 ± 2·7% (9), 5-HT 89·5 ± 2·5% (4), 5-HIAA 83·4 ± 1·9% (9). Results have not been corrected for losses.

Results

Effect of probenecid on the concentration of HVA and DOPAC in the nucleus basalis of the pigeon

The concentration of HVA in the nucleus basalis, which is the region of the brain richest in DA and 5-HT (Juorio & Vogt, 1967), was 0·79 ± 0·03 μg/g (twenty-three observations) and that of DOPAC 0·17 ± 0·01 μg/g (twenty-one observations). Figure 1 shows the time-course of the effect of probenecid on these two acidic dopamine metabolites. Probenecid (200 mg/kg, injected at time zero) increased the amount of both acids. The rise in HVA was significant ($P<0·001$) half an hour after the injection of probenecid and the increase in DOPAC 1·5 hr after the injection ($P<0·001$). The maximal concentrations of both acids were observed 4 hr after probenecid administration. At this time the HVA concentration had risen to 4 to 5 times the control value and the concentration of DOPAC had doubled. Six hours after the probenecid injection the concentrations of both acids were decreasing. At 10 hr the concentration of DOPAC had returned to normal values, whereas that of HVA remained elevated a little longer.

Figure 2 shows the changes in HVA and DOPAC concentrations in the nucleus basalis in response to different doses of probenecid. The observations were made 4 hr after drug injection. A dose of 50 mg/kg of probenecid increased the HVA concentration by about 40% ($P<0·001$) and 100 mg/kg increased it threefold. The concentration of DOPAC was significantly ($P<0·001$) increased after probenecid 100 mg/kg. The highest dose of probenecid used, 400 mg/kg, increased both HVA and DOPAC concentrations more than the dose of 200 mg/kg, but the difference

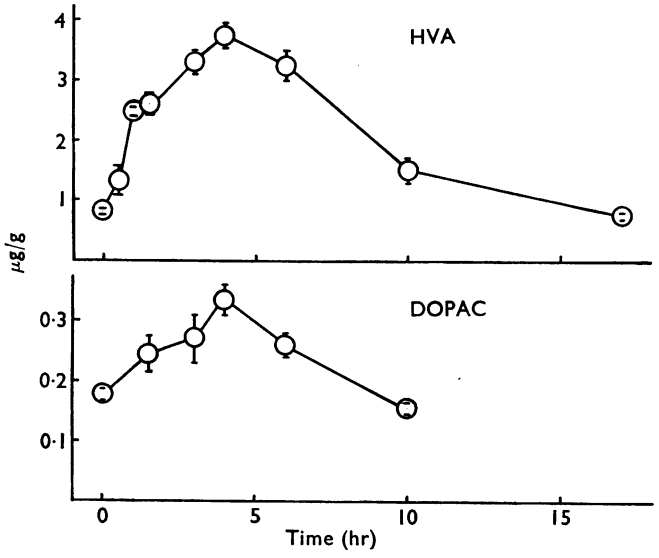

FIG. 1. Time course of the effect of probenecid (200 mg/kg intramuscularly) on the concentration (μg/g) of HVA and DOPAC in the nucleus basalis of the pigeon. Each point represents the mean of at least four estimations. Bars indicate S.E. of the mean. Note that the scales on the ordinates differ by a factor of 10.

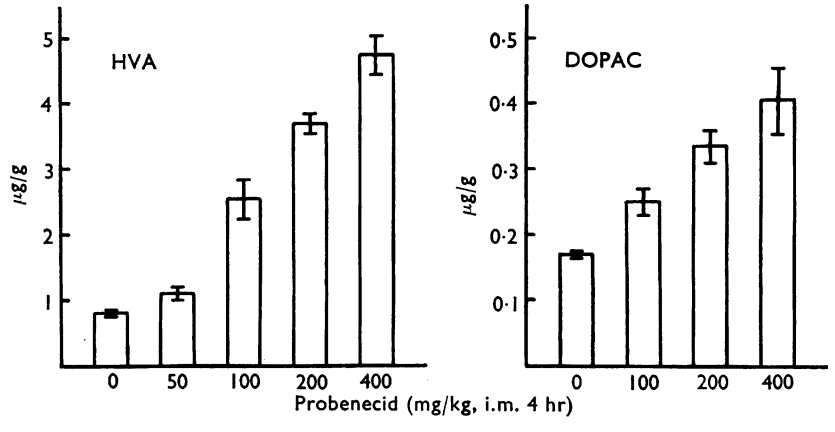

FIG. 2. Dose-response relation of the effect of probenecid on the concentration (μg/g) of HVA and DOPAC in the nucleus basalis of the pigeon. The columns represent the mean of at least four experiments. Bars indicate S.E. of the mean. Note that the scales on the two ordinates differ by a factor of 10.

TABLE 1. *Effect of reserpine injected intramuscularly on the HVA and DOPAC content (μg/g; mean ± S.E.) of the nucleus basalis of the pigeon*

Dose of reserpine (mg/kg)	Time elapsed since injection (hr)	HVA	DOPAC
None	—	0·79±0·032 (32)	0·17±0·006 (21)
1	6	0·37±0·033 (5)*	0·15±0·013 (5)
2	1	0·71±0·050 (5)	—
2	2	0·70±0·068 (4)	0·14±0·009 (5)
2	6	0·40±0·041 (6)*	0·11±0·016 (3)*
2	24	0·25±0·064 (3)*	0·12±0·015 (3)*

Number of experiments in brackets.
* Significantly different ($P<0·01$) from control value.

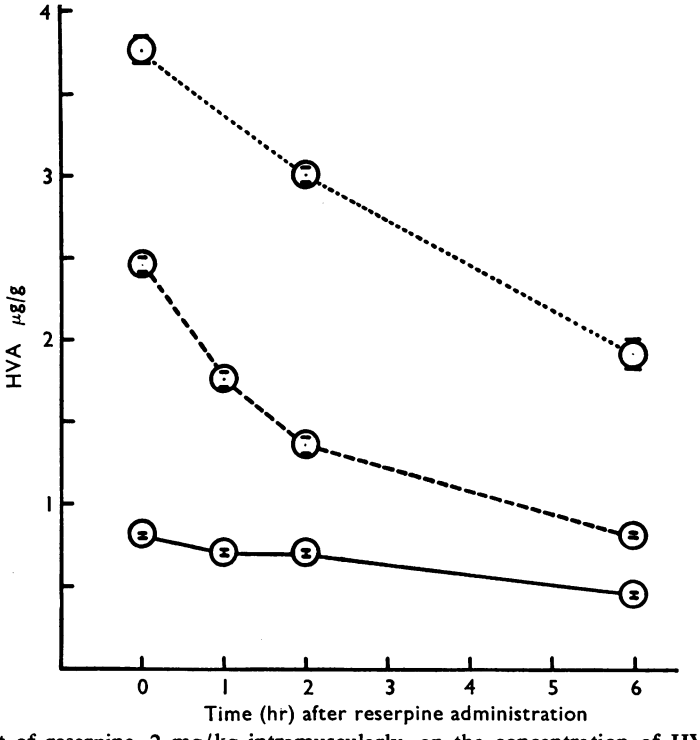

FIG. 3. Effect of reserpine, 2 mg/kg intramuscularly, on the concentration of HVA (μg/g) in the nucleus basalis of the pigeon at different times before or after probenecid, 200 mg/kg, injected intramuscularly. Reserpine alone, ○——○; probenecid 1 hr before decapitation, ○– – –○; probenecid 4 hr before decapitation, ○····○. Each point represents the mean of at least four experiments; small bars indicate the S.E. of the mean.

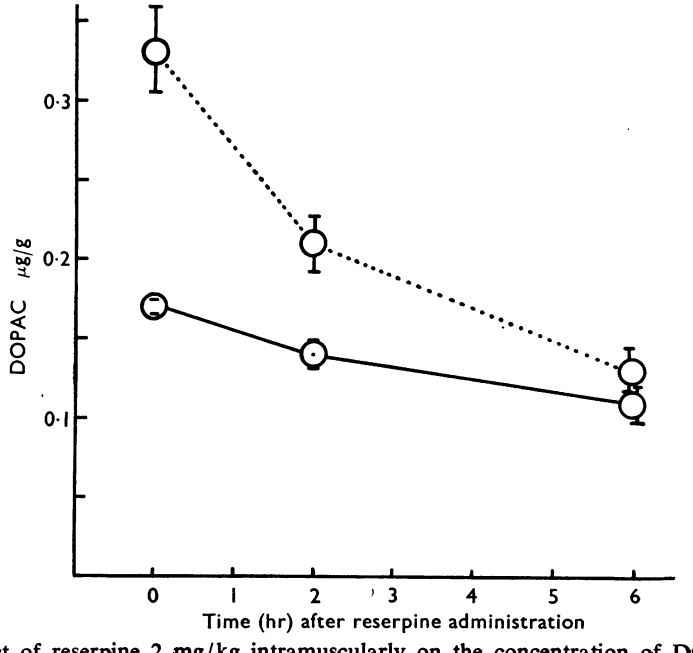

FIG. 4. Effect of reserpine 2 mg/kg intramuscularly on the concentration of DOPAC (μg/g) in the nucleus basalis of the pigeon injected intramuscularly with probenecid 200 mg/kg 4 hr before decapitation. Reserpine alone, ○——○; reserpine with probenecid, ○····○. Each point represents the mean of at least four experiments; bars indicate S.E. of the mean.

between doses was only significant ($P<0{\cdot}001$) for the rise in HVA. The values of HVA 4 hr after probenecid 200 mg/kg were of the same order of magnitude as those seen 1 hr after an intravenous injection of L-DOPA 100 mg/kg, and which amounted to HVA 4·5 μg/g and DOPAC 2·6 μg/g.

Effect of reserpine with and without probenecid on the HVA, DOPAC and 5-HIAA content of the nucleus basalis of the pigeon

Reserpine treatment decreased the concentrations of both HVA and DOPAC in the nucleus basalis (Table 1). The greatest falls were after 6 and 24 hr.

When reserpine was combined with probenecid 200 mg/kg (Figs. 3 and 4), it was found to lower the concentration of the acids within an hour of injection. By preventing the removal of HVA with probenecid, the reduction produced by reserpine became more conspicuous and was significant for a period of 6 hr (Fig. 3). The effect increased the longer the reserpine had been acting. Similar but smaller changes were found in the concentration of DOPAC (Fig. 4).

The effect of probenecid and reserpine on the 5-HIAA in the nucleus basalis is shown in Fig. 5. Both reserpine (2 mg/kg) and probenecid (200 mg/kg), given on their own, significantly ($P<0{\cdot}001$) increased the concentration of 5-HIAA 1 hr after administration. When both drugs were given together, a further rise in the concentration of 5-HIAA was observed. Probenecid caused no change in 5-HT concentration: 4 hr after administration of 200 mg/kg intramuscularly the amount of 5-HT in the nucleus basalis was $1{\cdot}11 \pm 0{\cdot}11$ μg/g, while the corresponding control value was $1{\cdot}29 \pm 0{\cdot}02$ μg/g.

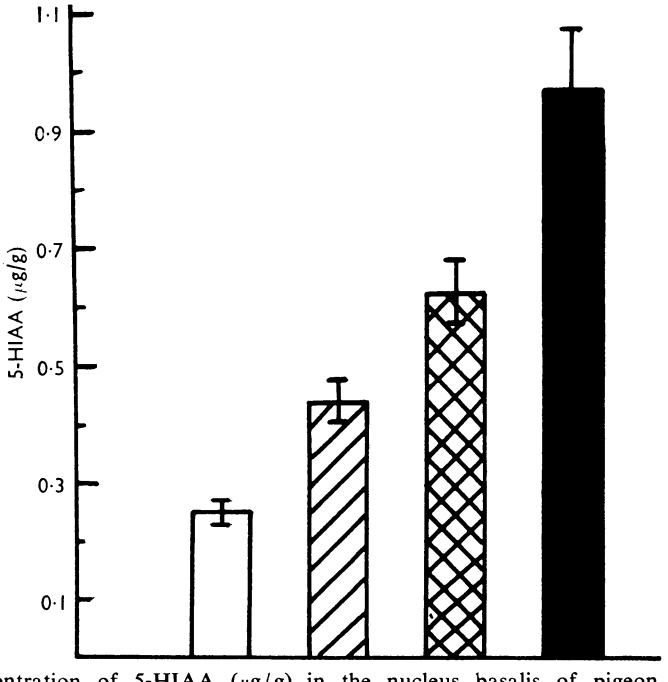

FIG. 5. Concentration of 5-HIAA (μg/g) in the nucleus basalis of pigeon brain. Clear column: controls; shaded column: 1 hr after intramuscular injection of reserpine 2 mg/kg; cross-hatched column: 1 hr after probenecid 200 mg/kg; black column: 1 hr after injection of both drugs. Standard errors of mean drawn as bars. Minimum number of experiments, four.

Effect of pargyline and reserpine on the dopamine content of the nucleus basalis of pigeon

Reserpine rapidly decreased the concentration of dopamine in the nucleus basalis (Table 2). Pargyline (100 mg/kg intramuscularly 17 hr before death) slightly increased the concentration of dopamine in this tissue. The loss of dopamine from the nucleus basalis caused by reserpine was partially prevented by pargyline, but the longer the reserpine treatment, the smaller was this effect (Table 2).

Effects on the behaviour and temperature of pigeons

Pigeons treated with probenecid alone had bouts of shuddering and regurgitated their food 15 to 30 min after the injection. Reserpine alone produced the same effects, but injection of saline did not. After probenecid 50 mg/kg the pigeons were slightly more quiet than the controls, and after 100 mg/kg and 200 mg/kg they were clearly sedated ; the effect was evident 0·5 hr after the injection. The degree of sedation was dose dependent. After a dose of 400 mg/kg the pigeons lay on their breast in the cage, with closed eyes, but they could be awakened and some of them were in fact hypersensitive to touch and ran wildly around the cage if disturbed.

The maximal sedative effect of probenecid occurred about 3 to 4 hr after the injection, but some sedation was still noticeable after 6 hr. No abnormal behaviour was detectable after 10 hr.

Reserpine 1 or 2 mg/kg caused more sedation than probenecid ; yet it caused only a slight drop in cloacal temperature, whereas probenecid had a pronounced hypothermic effect (Fig. 6).

When probenecid and reserpine were given together, the birds were heavily sedated and almost cataleptic. They were not able to fly and remained in the position in which they were put. When the higher reserpine dose (2 mg/kg) was combined with 200 mg/kg of probenecid, some of the birds died 4 to 5 hr after administration of the drugs. The effect of the combination of these drugs on the cloacal temperature was not greater than that of probenecid alone (Fig. 6). The figure also indicates that there was no correlation between fall in body temperature and rise in cerebral HVA.

Treatment with pargyline sedated the pigeons and their feathers were fluffed up, an effect not seen with the other drugs. When pargyline and reserpine were given together the pigeons were more sedated than with either of these drugs alone and they did not try to fly. Some of them were also cataleptic and remained in the position in which they were put.

Pargyline (100 mg/kg, 17 hr) lowered the temperature of the birds by about 3° C. When reserpine was also injected the temperature fell about 1° C further.

TABLE 2. *Dopamine content (μg/g; mean* ±S.E.) *of the nucleus basalis of the pigeon after treatment with pargyline* (100 *mg/kg* 17 *hr before the end of the experiment*) *and with reserpine* 1 *mg/kg*

Duration of action of reserpine (hr)	Dopamine	
	No pargyline	Pargyline
0	2·95±0·17 (13)	3·52±0·36 (7)
0·5	1·17±0·47 (5)	2·89±0·23 (6)
1	0·70±0·21 (3)	2·89±0·48 (5)
4	0·25, 0·16 (2)	1·85±0·26 (6)
6	0·08±0·005 (3)	1·41±0·08 (4)

Number of experiments in brackets.

Behaviour of pigeons and chicks after injection of HVA and other acids

The object of these experiments was to see whether the sedation seen after probenecid could be interpreted as an effect of accumulation of HVA or other acids in the brain. Two adult pigeons were injected under ether anaesthesia with HVA intracisternally. One was given 67 μg and the other 300 μg of HVA. Recovery from the effects of anaesthesia took 5–10 min. No changes were observed in behaviour of these pigeons when compared with controls which were injected with saline in similar conditions.

To avoid the effects of anaesthesia and to permit uniform penetration of the drug into the brain further studies were carried out on newly hatched chicks, which were injected intravenously with various doses of HVA, DOPAC, 5-HIAA, 3,4-dimethoxyphenylacetic acid and sodium γ-hydroxybutyrate. HVA was injected in doses of 0·67, 1·35, 2·7, 5·4 and 8·1 mg to chicks weighing 39 to 43 g; the doses of DOPAC were 1·35 to 5·4 mg, of 5-HIAA 5·4 mg, and of dimethoxyphenylacetic acid 5·4 mg. None of these injections produced changes in behaviour; short periods of eye closure and drooping of the head occurred in all chicks, including some which were given 0·9% sodium chloride solution or not injected at all. In contrast, injection of sodium γ-hydroxybutyrate in doses of 1·4 to 4·2 mg caused immediate paralysis followed by prostration and eye closure, and the effect was dose dependent.

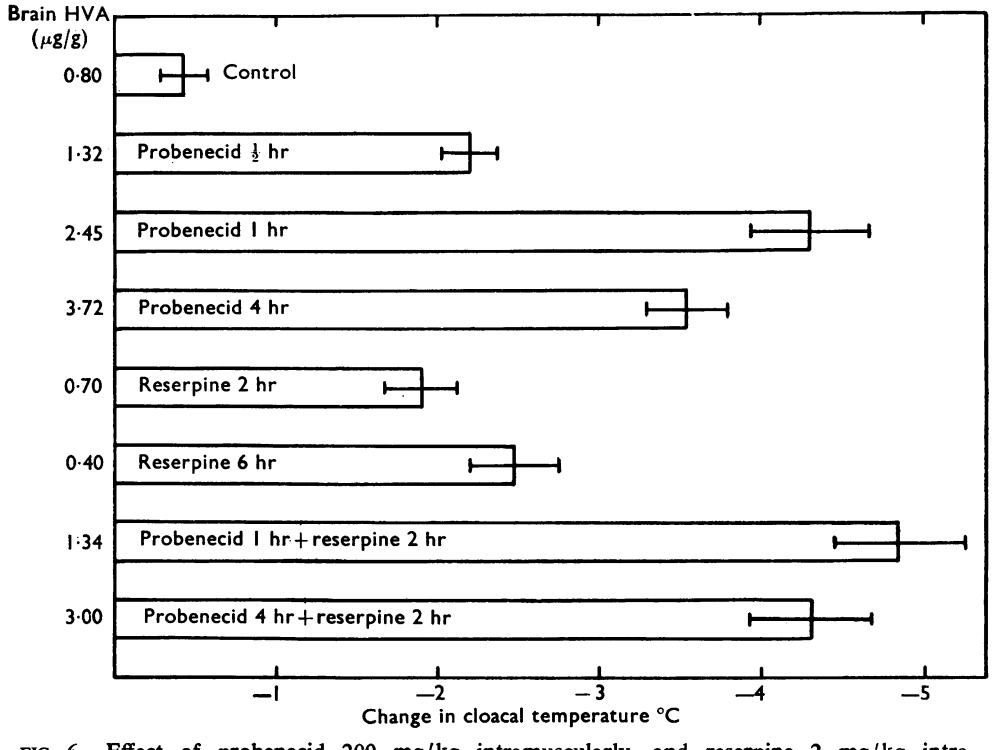

FIG. 6. Effect of probenecid 200 mg/kg intramuscularly, and reserpine 2 mg/kg intramuscularly, single or combined, on the cloacal temperature of pigeons. Changes in degrees C ± S.E.; each column is the mean of at least five measurements. The corresponding concentrations of brain HVA are seen on the left.

HVA concentration in chicken brain; effect of probenecid and of intravenous administration of large quantities of HVA

Although the preceding experiments appeared to exclude the accumulation of HVA, DOPAC and 5-HIAA as the cause of sedation produced by probenecid, it appeared necessary to check how much of these acids had entered the brain after intravenous injection and to compare the concentrations obtained with those occurring in the chick after probenecid.

Estimations were made on the whole of the nucleus basalis when it was discovered that the concentration of both acid metabolites of DA was extremely low in the chicken. In the adult bird the concentration of HVA was $0{\cdot}06 \pm 0{\cdot}006$ μg/g (six estimations); similar amounts were found in the nucleus basalis of chicks aged 2 days, 4 days, 1 week and 2 weeks. Probenecid 200 mg/kg intramuscularly increased the concentration in 1·5 hr to $0{\cdot}24 \pm 0{\cdot}027$ μg/g; after 4 hr it was $0{\cdot}10 \pm 0{\cdot}003$ μg/g. The probenecid-treated chickens were quieter than the controls. For the DOPAC estimations the nucleus basalis region of two or three chicks 8 days old was pooled and the value obtained was $0{\cdot}053 \pm 0{\cdot}002$ (six estimations), thus nearly as high as that for HVA.

The amount of HVA in brain and plasma after intravenous administration of approximately 100 mg/kg of HVA was studied in 1, 2, 4 and 7 days old chicks. HVA in the nucleus basalis of 1 day old chicks was over 10 μg/g, more than twice

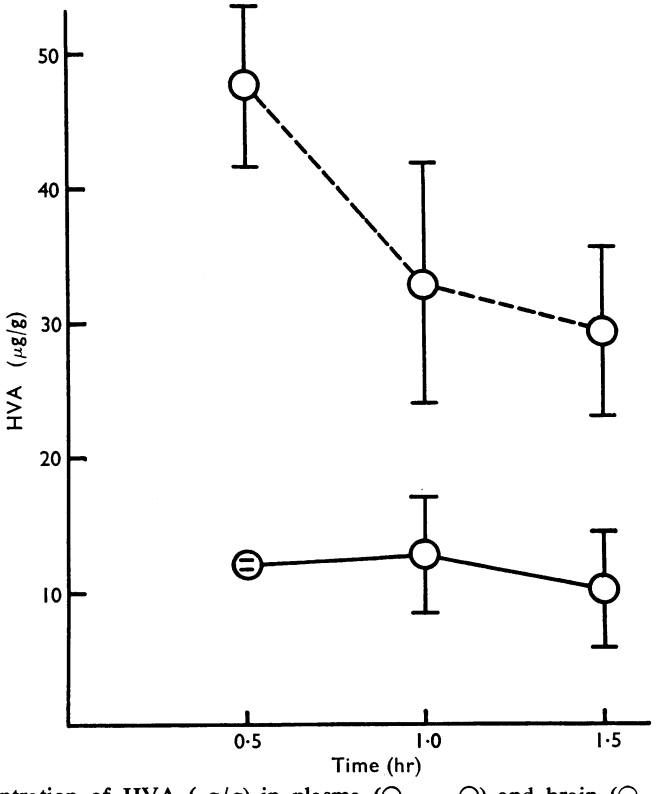

FIG. 7. Concentration of HVA (μg/g) in plasma (○– – –○) and brain (○——○) of 1 day old chicks at various times after intravenous injection of HVA 100 mg/kg (approx.). Bars indicate S.E. of the mean. Each point is the mean of at least three experiments. The whole nucleus basalis was used for the estimations in brain.

the highest concentration seen after probenecid, for the period of 0·5 to 1·5 hr after intravenous injection of the acid. During this interval the plasma concentration fell to about 3 times the concentration in the brain (Fig. 7). In Fig. 8 HVA was estimated in chicks of different age 1 hr after the intravenous injection. Only in chicks 1–4 days of age did the brain concentration attain values comparable to those seen after the largest doses of probenecid. The concentration of HVA was 3–6 times higher in the plasma than in the brain. Assuming that, in the exsanguinated chick, blood left in the brain might have represented as much as 5% of total weight—a figure which is certainly too high—the contribution of blood-borne HVA to the total content of the brain can, at most, have been 30%.

Discussion

The concentrations of DA, HVA, 5-HT and 5-HIAA reported by Juorio & Vogt (1967) in the nucleus basalis of pigeon brain were confirmed. In addition, it was found that the concentration of DOPAC was only one-fifth of that of HVA. In the chicken, the concentration of HVA was only 10% of that found in the pigeon ; yet the DA content of the nucleus basalis is the same in the two species (Juorio & Vogt, 1967) ; thus the ratio DA/HVA is about 50 in chicken brain.

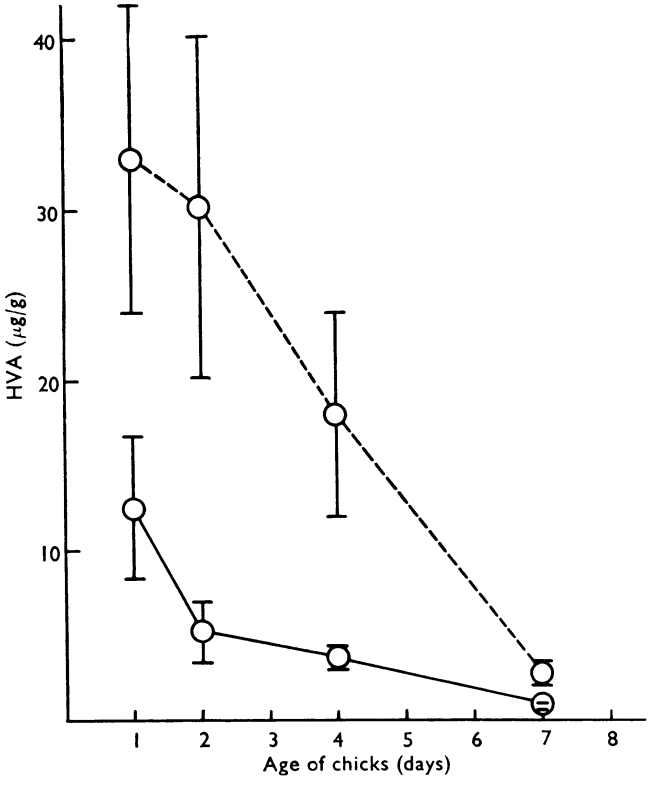

FIG. 8. Concentration of HVA in plasma (○- - -○) and brain (○——○) of chicks of different ages one hour after intravenous injection of HVA 100 mg/kg (approx.). Bars indicate S.E. of the mean. Minimum number of experiments, three. The whole nucleus basalis was used for the estimations in brain.

Probenecid

Probenecid increased the amount of the acidic amine metabolites HVA, DOPAC and 5-HIAA in the nucleus basalis of the pigeon brain. This indicates that in this species there is an active transport mechanism for the removal of these acids from brain. Probenecid also increased the amount of HVA in the nucleus basalis of the chicken brain ; thus a similar mechanism is present in the chicken. After probenecid, the maximum increase in HVA content of the nucleus basalis of the pigeon was 4 to 5 times the control value. This increase was even higher than that found in the small rodents (Sharman, 1966, 1967 ; Werdinius, 1966, 1967b, 1968). The ratio DA/HVA in the tissue fell below 1. In the chicken brain probenecid also increased the amount of HVA about four-fold ; however, owing to the low initial HVA concentration, there was still about 10 times as much DA than there was HVA in the tissue. This suggests that the rate of formation of HVA is much slower in the chicken than in the pigeon, provided all the HVA is removed from the brain by the probenecid-sensitive transport system.

Probenecid treatment doubled the DOPAC concentration in the nucleus basalis of the pigeon. Sharman (1969) did not see a significant increase in the concentration of DOPAC in mouse brain after probenecid. Thus it appears that in the pigeon, but not in the mouse, some DOPAC is removed from the brain by an active transport mechanism similar to that for HVA.

Reserpine

Andén, Roos & Werdinius (1964) found that in rabbits, reserpine treatment caused an increase in the amount of both deaminated and deaminated/O-methylated amine metabolites. This has been shown to occur also in other mammals, for example, mouse and rat (Sharman, 1967 ; Werdinius, 1967b, 1968). In the pigeon, Juorio & Vogt (1967) found that reserpine, while causing a decrease in the concentration of brain monoamines, did not raise the concentration of HVA, although that of 5-HIAA was increased.

In the present experiments no rise in HVA or DOPAC concentration was found after administration of reserpine, and 6 hr after the injection, when the amount of DA was at its lowest, HVA and DOPAC were significantly decreased. This decrease in the concentrations of acidic DA metabolites was more clearly demonstrated when the birds were treated with both reserpine and probenecid. By raising the concentration of these acids with probenecid, the falls produced by reserpine became larger and were significant within an hour of reserpine administration. These observations are interpreted as an interference by reserpine with the formation of acidic DA metabolites. In contrast, as could be expected from the experiments by Juorio & Vogt (1967) on reserpine, both this drug and probenecid increased the concentration of 5-HIAA as they do in mammals. Their effect was additive.

The mechanism of action of reserpine is supposed to be a block in the storage of amines in granules, and this leads to an increased enzymatic inactivation of amines by monoamine oxidase. Because in the pigeon brain the formation of acidic metabolites is decreased after reserpine, the interaction of a monoamine oxidase inhibitor, pargyline, with reserpine was studied. Pargyline itself did not significantly increase the DA content, but it prevented to some extent the decrease in DA content caused by reserpine. Therefore the mechanism of action of reserpine in

decreasing the amount of DA in pigeon brain does involve an increased destruction of DA by monoamine oxidase. In spite of this, the formation of acidic metabolites is depressed, and one may therefore suspect that there is an increased production of non-acidic metabolites. An alternative mechanism could be a depression of DA synthesis, and it is also possible that both modes of action are involved.

Behaviour

Pigeons and chickens were sedated by probenecid. The deepest sedation occurred at about the same time as the greatest increase in acidic amine metabolites in brain. However, no evidence was obtained that an accumulation in the brain of HVA or any of the other acids, achieved by intracisternal injection into the adult bird or intravenous injection into the young chick, produced any effect on behaviour. The measurement of HVA concentrations in plasma and brain after intravenous injection showed that HVA penetrated into the brain of young chicks in amounts comparable with those seen after administration of probenecid. Though the HVA in plasma was also high in such experiments, contamination with blood was only responsible for a fraction of the HVA present in brain tissue.

When probenecid and reserpine were given together, the depressant effect of reserpine was potentiated and some of the pigeons became cataleptic. This effect never occurred even after toxic doses of probenecid alone. On the other hand, the hypothermic effects of the two drugs were not additive.

Pargyline also sedated the pigeons, and when it was given with reserpine, this effect was more pronounced. As also seen by Watts, Mendez, Reilly & Krop (1969), the cloacal temperature fell in the pigeon after pargyline. It fell further when reserpine was given as well.

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