vitamin B₆ activities of these hydrazides, unless the bacterial cell is selectively permeable to isoniazid alone.

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The Blocking Activity of Sympathomimetic Amines on Adrenaline Hyperglycaemia

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Previous workers have found that while a di- or mono-hydroxybenzene ring must be present for a sympathomimetic amine to have hyperglycaemic activity, the analogous deoxy compound can still affect the blood pressure. In view of this, the effect on the hyperglycaemic and blood-pressure-raising activities of replacing the catechol or benzene ring of sympathomimetic amines by a pyridine ring was studied.

Ellis (1951) suggested that in amines with hyperglycaemic activity the side chain is responsible for the attachment to the enzyme complex concerned in adrenaline hyperglycaemia, whereas the catechol nucleus is the active grouping in the production of hyperglycaemia. Thus, on this hypothesis, it may be possible for a compound having a side chain similar to that of adrenaline to attach itself, and, while exerting no hyperglycaemic effect owing to the lack of the appropriate aromatic structure, block the hyperglycaemic effect of adrenaline administered subsequently. In agreement with this hypothesis Ellis found that ephedrine did, in fact, largely

inhibit the adrenaline effect in rats, and this was confirmed for rabbits by Komrad & Loew (1951).

Some information on how far this ephedrine-blocking effect is dependent on an *N*-methylated hydroxyisopropylamine side chain attached to a benzene nucleus has now been obtained by using norephedrine and its pyridine analogue and the pyridine analogue of α -aminopropiophenone. Synthetic methods for preparing these two pyridine analogues, namely 3-(2-amino-1-hydroxypropyl)-pyridine (IV) and 3-(2-amino-1-oxopropyl)pyridine (II) respectively, have been developed.

The 3-(2-amino-1-oxopropyl)pyridine was prepared by the action of nicotinic anhydride on α -benzamidopropionic acid, according to Dakin & West (1928), followed by a hydrolysis of the benzamido group. In order to obtain the corresponding hydroxypropyl derivative, which was prepared by catalytic reduction of the ketone, it was necessary first to reduce the benzamido derivative and then hydrolyse, since hydrogenation of the free amine could not be achieved without simultaneous

reduction of the pyridine ring. The corresponding ketone from *isonicotinic* anhydride could not be obtained, as hydrolytic removal of the benzoyl group was accompanied by deamination.

In an attempt to account for the relatively low yields in the Dakin & West reaction the effect of heating the intermediate oxazolone from benzamido-propionic acid alone in the presence of the basic catalyst was determined. This led to the isolation of a substance $C_{20}H_{18}O_4N_2$, presumably 3-benzamido-1-benzoyl-2:4-dioxo-3:5-dimethylpyrrolidine (cf. Lawson, 1956).

EXPERIMENTAL

Synthetic methods

3-(2-Benzamido-1-oxopropyl)pyridine (I). Nicotinic anhydride (23 g., 0.1 mole) prepared by the method of Schrecker & Maury (1954) was mixed intimately with α -benzamido-2-methylpropionic acid (7.7 g., 0.04 mole) and anhydrous 3-methylpyridine (17.4 ml., 0.18 mole) was added. The mixture was heated under nitrogen at 90° with occasional shaking until carbon dioxide evolution had ceased (about 4.5 hr.). The bulk of the picoline was removed by distillation under reduced pressure and the residue refluxed with water for 1 hr. The cooled solution was made alkaline with aqueous sodium bicarbonate and then extracted with chloroform. The chloroform on evaporation left a dark-coloured residue which dissolved in hot ethanol, and after addition of light petroleum (b.p. 40–60°) the white crystalline ketone came down on standing at 0° (yield 53%). Recrystallization from ethanol–light petroleum gave needles, m.p. 125–126° (Found: C, 70.6; H, 5.4; N, 10.7. $C_{15}H_{14}O_4N_2$ requires C, 70.8; H, 5.5; N, 11.0%). The use of 2:4:6-collidine as basic catalyst decreased the yield to 28%.

3-(2-Amino-1-oxopropyl)pyridine dihydrochloride (II). The ketone (I) (2 g.) was heated under reflux with 3*N*-hydrochloric acid (50 ml.) for 1 hr. The benzoic acid was removed by filtration of the cooled solution, and evaporation under reduced pressure left the *amine dihydrochloride*, which was recrystallized from aqueous ethanol, m.p. 221° (decomp.) after darkening (yield 30%) (Found: C, 43.8; H, 5.6; N, 12.4; Cl, 30.3; $C_8H_{10}ON_2 \cdot 2HCl$ requires C, 43.1; H, 5.4; N, 12.6; Cl, 31.8%). The *dipicrate*, forming prismatic needles from water, had m.p. 168° (decomp.) (Found: C, 39.3; H, 2.4; N, 18.8. $C_{20}H_{18}O_5N_4$ requires C, 39.5; H, 2.6; N, 18.4%). The hydrochloride on treatment with 3*N*-sodium hydroxide evolved ammonia, and an unidentified orange-coloured substance was precipitated. This on crystallization from ethanol–light petroleum gave colourless prisms, m.p. 126° (Found: C, 70.5; H, 5.5; N, 10.3%).

3-(2-Benzamido-1-hydroxypropyl)pyridine (III). 3-(2-Benzamido-1-oxopropyl)pyridine (5 g.) was dissolved in ethanol (300 ml.) and hydrogenated in the presence of Adams platinum-black catalyst (1 g.) for 2 hr. The residue left after removal of the catalyst and solvent was recrystallized from ethanol or water, and had m.p. 160° (yield, 90%) (Found: C, 69.9; H, 6.2. $C_{15}H_{16}O_3N_2$ requires C, 70.3; H, 6.25%). The *picrate*, crystallizing as needles from ethanol, had m.p. 179° (Found: C, 52.6; H, 4.0. $C_{21}H_{18}O_6N_4$ requires C, 52.8; H, 3.9%).

3-(2-Amino-1-hydroxypropyl)pyridine (IV). The hydroxy compound (III) (2 g.) was hydrolysed by boiling under reflux with 20% (w/v) aqueous hydrochloric acid (50 ml.). After removal of the benzoic acid by filtration and the hydrochloric acid by evaporation under reduced pressure, the residue of *amine dihydrochloride* (yield 82%) was recrystallized from ethanol as prisms, m.p. 228–229° (Found: C, 42.6; H, 6.4; N, 12.6. $C_8H_{12}ON_2 \cdot 2HCl$ requires C, 42.6; H, 6.2; N, 12.4%). The *dipicrate*, forming prismatic rods from water, had m.p. 202° (decomp.) (Found: C, 37.3; H, 3.5. $C_{20}H_{18}O_5N_4 \cdot 2H_2O$ requires C, 37.2; H, 3.6%).

4-(2-Benzamido-1-oxopropyl)pyridine. The isonicotinic anhydride used was prepared as described by Schrecker & Maury (1954) except that the reaction between the oxaly chloride and potassium isonicotinate was carried out at room temperature for the first hour and at 80° for the second hour. The reaction with benzamidopropionic acid was carried out as for the nicotinic analogue (above). The yield was 23% as prisms from ethanol, m.p. 119–120° (Found: C, 70.8; H, 5.4; N, 10.7. $C_{15}H_{14}O_2N_2$ requires C, 70.8; H, 5.5; N, 11.0%). This substance could not be hydrolysed successfully to give the corresponding amine. Hydrolysis of the benzoyl group with 3N- or N-hydrochloric acid was accompanied by deamination of the resulting amine.

When collidine was used as the basic catalyst the only material isolated was isonicotinic anilide, m.p. 171°, formed from aniline present as an impurity in the collidine (Found: C, 72.9; H, 5.3; N, 13.8. $C_{15}H_{10}ON_2$ requires C, 72.7; H, 5.1; N, 14.1%).

Effect of heating 4-methyl-2-phenyloxazolone in picoline. α -Benzamidopropionic acid (2.5 g.) was dissolved in warm acetic anhydride and the anhydride removed by repeated distillation under reduced pressure after addition of xylene. The gummy residue, oxazole, was heated at 85° under nitrogen for 2 hr. with dry 3-methylpyridine (5 ml.). After removal of the picoline the light-yellow oil was treated with aqueous sodium bicarbonate and extracted with chloroform. The residue from the chloroform was crystallized from boiling ethanol to give 3-benzamido-1-benzoyl-2,4-dioxo-3,5-dimethylpyrrolidine (V), forming felted needles, m.p. 243° (yield 35%) (Found: C, 68.6; H, 5.1; N, 8.1. $C_{20}H_{18}O_4N_2$ requires C, 68.6; H, 5.1; N, 8.0%). Hydrolysis with ethanolic 2N-hydrochloric acid for 15 min. gave 3-benzamido-2,4-dioxo-3,5-dimethylpyrrolidine, m.p. 218° (from ethanol-light petroleum) (Found: C, 63.7; H, 5.5; N, 10.8. $C_{13}H_{14}O_3N_2$ requires C, 63.4; H, 5.7; N, 11.4%). When the oxazolone derivative was heated in collidine instead of picoline the pyrrolidine derivative was obtained in less yield and the aniline present as impurity gave α -benzamidopropionanilide, m.p. 172° (Found: C, 71.4; H, 5.9; N, 10.3. $C_{16}H_{16}O_3N_2$ requires C, 71.6; H, 6.0; N, 10.4%).

Biological methods

Male albino rats (205–314 g. body wt.) were used. They were not deprived of food. Blood samples were obtained from the tail of the unanaesthetized animal. The blood-sugar determinations were carried out by Somogyi's (1945) method. All injections were given intraperitoneally, the solvent being 0.9% sodium chloride buffered where necessary to pH 6.6 with sodium bicarbonate. The volume of solution injected was in all cases 2.5 ml./kg. body wt. The animals were allowed at least 6 days rest between experiments.

RESULTS

The effects of the various compounds on blood-sugar levels are shown in Table 1. The blood sugar was at the normal level 30 min. after the injection of 3-(2-amino-1-oxopropyl)pyridine and of 3-(2-amino-1-hydroxypropyl)pyridine. Neither of these compounds affected significantly the hyperglycaemia produced by adrenaline ($P > 0.1$). Adrenaline hyperglycaemia was significantly reduced by prior injection of either ephedrine ($P < 0.001$) or norephedrine ($P 0.01$ – 0.001), and the two adrenaline-hyperglycaemia-blocking effects were not significantly different from one another ($P > 0.1$).

Ephedrine, norephedrine and 3-(2-amino-1-oxopropyl)pyridine produced similar effects on the behaviour pattern of the rats. They caused hyperexcitability. Salivation was very marked after administration of ephedrine and norephedrine. With 3-(2-amino-1-hydroxypropyl)pyridine there were no apparent toxic symptoms apart from a slight paralysis of the hind leg in some animals.

When injected into anaesthetized cats, both pyridine derivatives exhibited sympathomimetic activity. 3-(2-Amino-1-oxopropyl)pyridine (II)

Table 1. *Effect of DL-3-(2-amino-1-oxopropyl)pyridine dihydrochloride and DL-3-(2-amino-1-hydroxypropyl)pyridine dihydrochloride on blood-sugar level, and the effect of these compounds and of L-ephedrine hydrochloride and DL-norephedrine hydrochloride on L-adrenaline hyperglycaemia*

Expts. 1–6 were carried out on each of a group of fifteen rats except where shown; Expts. 7 and 8 were done on each of a separate group of eight rats. In Expts. 4–8, 0.1 mg. of adrenaline/kg. body wt. was injected immediately after the second blood sample had been taken at 30 min.

The relative changes of blood-sugar level at various times are expressed as a percentage of the blood-sugar value at zero time (mean values $\pm$ S.E.).

Expt. no.	Drug and dosage (mg. of base/kg. body wt.) administered at zero time	Relative change of blood-sugar level with time (min.)		
		30	60	90
1	0.9% NaCl	103 $\pm$ 3	97 $\pm$ 2	—
2	3-(2-Amino-1-oxopropyl)pyridine	95 $\pm$ 3	98 $\pm$ 4	—
3	Adrenaline	0.1	168 $\pm$ 6	—
4	3-(2-Amino-1-oxopropyl)pyridine	25	104 $\pm$ 2	157 $\pm$ 4
5	Ephedrine	25	100 $\pm$ 3*	117 $\pm$ 5†
6	Norephedrine	25	104 $\pm$ 7‡	132 $\pm$ 9‡
7	0.9% NaCl	—	100 $\pm$ 2	148 $\pm$ 6
8	3-(2-Amino-1-hydroxypropyl)pyridine	25	102 $\pm$ 2	152 $\pm$ 6

* Mean of fourteen rats.

† Mean of thirteen rats.

‡ Mean of ten rats.

produced a rise in blood pressure, probably through an ephedrine-like action. Its potency was about a fifth of that of ephedrine and it also caused tachyphylaxis. On the other hand, the sympathomimetic activities of 3-(2-amino-1-hydroxypropyl)pyridine (IV) resembled more those of adrenaline and noradrenaline. In addition, this compound stimulated autonomic ganglia.

DISCUSSION

It has been shown that norephedrine is as effective as ephedrine in reducing adrenaline hyperglycaemia. It is possible therefore that the failure of 3-(2-amino-1-oxopropyl)pyridine and 3-(2-amino-1-hydroxypropyl)pyridine to act similarly is not due to the absence of an *N*-methyl group in the side chain. Nor can the lack of activity of these compounds be attributed to the isopropylamine side chain that is present in all four amines. Since the hydroxypropyl derivative differs from norephedrine only in the substitution of a pyridine for a benzene ring, it is evident that some alterations to the ring moiety of ephedrine analogues interfere with their adrenaline-hyperglycaemia-blocking activity.

Although norephedrine has no hyperglycaemic activity, that of its catechol analogue, cobeprin, is very marked (Schaumann, 1931; Anderson & Chen, 1934). In the present work the pyridine analogue of cobeprin and norephedrine, 3-(2-amino-1-hydroxypropyl)pyridine, has been shown to exhibit no hyperglycaemic activity. While the substitution of a pyridyl for a dihydroxyphenyl radical abolished

the hyperglycaemic effect, the blood-pressure reaction of the ephedrine type of amine was replaced by one more characteristic of adrenaline and noradrenaline. The effect of substituting the side chain at the 4-position on the pyridine ring could not be determined owing to the difficulty of obtaining the required amines.

SUMMARY

1. Ephedrine and norephedrine significantly reduced adrenaline-induced hyperglycaemia in rats.
2. 3-(2-Amino-1-oxopropyl)pyridine and 3-(2-amino-1-hydroxypropyl)pyridine did not change the blood-sugar level of rats or affect the hyperglycaemia produced by adrenaline. Both compounds exhibited sympathomimetic activity.

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The Synthesis of Lipids in the Livers of Rats Treated with Pituitary Growth Hormone

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Studies on the carcass composition of rats treated with pituitary growth hormone (Lee & Schaffer, 1934; Young, 1945; Greenbaum, 1953) have led to the conclusion that the hormone exerts a profound influence on the course of fat metabolism.

Growth hormone injected into the intact rat stimulates fat catabolism in the liver, but this alone is not considered sufficient to account for the decline in total body fat which is also observed. A stimulation of the extrahepatic utilization of fat has been

postulated as well as the increased liver catabolism (Greenbaum & McLean, 1953*b*). In addition to the increased rate of fat catabolism it might be expected that there would also be a reduced rate of fat synthesis. Such an inhibition of fat synthesis has already been reported by Welt & Wilhelmi (1950-51) and by Brady, Lukens & Gurin (1951). On the other hand, Allen, Medes & Weinhouse (1956) were unable to obtain a consistent inhibition and were not satisfied that growth hormone has a direct