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COMPARISON OF THE ACTIONS OF CENTRALLY AND PERIPHERALLY ADMINISTERED CLONIDINE AND GUANFACINE IN THE RABBIT: INVESTIGATION OF THE DIFFERENCES

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1 Guanfacine was administered intravenously to rabbits and produced a dose-dependent lowering of blood pressure.

2 Clonidine and guanfacine, administered to rabbits intravenously (30 μg/kg and 300 μg/kg respectively) and intracisternally (3 μg/kg and 12 μg/kg respectively) caused a similar degree of hypotension, apparently of central origin.

3 Saliva flow *in vivo* was estimated. Clonidine (30 μg/kg, i.v.) caused a significant decrease in salivation ($P<0.05$) for the first 50 min after injection. Guanfacine caused a significant fall ($P<0.05$) only at 50 and 180 min after injection.

4 Apparent partition coefficients for an octanol/buffer system at pH 7.4 for clonidine and guanfacine were 5.4 and 21.2 respectively.

5 Measurement of guanfacine levels concurrently in both plasma and brain showed that guanfacine had higher brain than plasma levels and that the brain levels were fairly constant over the 3 h measured. Brain:plasma ratios were 2.1:1, 5.3:1 and 13.6:1 after 15, 90 and 180 min respectively.

6 These results suggest that the long duration of action of guanfacine is due to its persistence at its central site of action.

Introduction

Guanfacine (BS 100-141:N-amidino-2-(2,6-dichlorophenyl acetamide), is a centrally acting antihypertensive drug with a pharmacological profile broadly similar to that of clonidine (Bream, Lavener, Picard, Scholtysik & White, 1975; Scholtysik, Lauener, Eichenberger, Burk, Saltman, Muller, Schweinitzer & Waite, 1975; Dubach, Huwyler, Radielovic & Singelsen, 1977). Differences between the two drugs which may be of clinical significance are that guanfacine has a longer duration of action and fewer side effects (Zamboulis, Reid & Hamilton, 1978). In this study we have compared the actions of guanfacine and clonidine in the rabbit. We administered both drugs by central and peripheral routes, compared their effects on blood pressure, heart rate and salivation and also distribution of guanfacine between blood and brain.

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Methods

Animals and drugs

Male New Zealand white rabbits of 2.5–3.5 kg were used throughout. Clonidine hydrochloride (Boehringer Ingelheim, Bracknell, Berks) and guanfacine hydrochloride (Sandoz Ltd, Feltham, Middlesex) were used, made up in 0.9% w/v sodium chloride for i.v. injection and in sterile 0.9% w/v sodium chloride for i.c. injection. Control animals were given the same volume of vehicle. For calculation of the partition coefficient ^{14}C-labelled drugs were used.

Administration of drugs

Administration was by the intravenous (i.v.) or intracisternal (i.c.) routes. Intravenous administration was by a disposable needle (Butterfly, Abbot Ltd) in the marginal ear vein. Intracisternal administration was by transcutaneous freehand injection into the cisterna magna of the rabbit, anaesthetized with pentobarbitone (Chalmers & Wurtman, 1971). The injection volume was 1 ml for the i.v. route and 100 μl for i.c.

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Measurement of blood pressure and heart rate

Blood pressure was measured directly in the unrestrained animal by cannulation of the central artery of the ear under local anaesthesia (Chalmers & Reid, 1972). Animals were then left for at least an hour or until their mean arterial pressure (MAP) was stable, whichever was the longer. Blood pressure was measured with a Statham pressure transducer and a Devices M2 recorder. Mean arterial pressure was determined electronically and heart rate was calculated from the pulsatile wave.

Salivation

The method of measurement is a variation of that reported by Dollery, Davies, Draffan, Dargie, Bean, Reid, Clare & Murray (1976), in which pre-weighed cotton wool dental rolls were placed in the mouth for a fixed period of time, then removed and re-weighed. We have adopted this method for the rabbit. The animal was placed in a restraining collar, then a 21 gauge butterfly needle was placed in the marginal vein of the ear half an hour before injection of drug or vehicle. At the time of measurement one of a pair of weighed dental rolls (Oratex No. 1), held in a pair of artery forceps, was inserted in the side of the mouth through a gap in the dentition. After being held in position for 30 s it was removed, and, after a 30 s pause, the other roll was inserted, again for 30 s. Both rolls were placed in airtight containers and re-weighed at the end of the experiment. The difference in weights before and after insertion was taken as an indication of salivary flow.

Octanol-buffer apparent partition coefficient

Ten ml of octanol was vortexed for 5 min with 10 ml of 0.1 M phosphate buffer (pH 7.4) to which a millimolar solution of ^{14}C-labelled clonidine or guanfacine had been added. The two layers were then separated by centrifugation (5 min, 2000 rev/min) and a 1 ml aliquot of each layer taken for scintillation counting. The proportion of the counts between the two layers was taken as the apparent partition coefficient.

Brain-plasma ratio

Guanfacine 300 μg/kg was injected i.v. into three groups of five rabbits each. The first group was killed 15 min after the injection, the second 90 min after injection and the third after 180 min. Animals were killed by injection of sodium pentobarbitone, a sample of blood being taken by cardiac puncture, and the whole brain was immediately removed and deep frozen. The blood was centrifuged (2000 rev/min, 15 min, 4°C) and the plasma deep frozen until assayed.

The assay was by a modification of the GC-MS method of Laplanche & Morin (1978) using $^{13}C^{15}N_3$ guanfacine as internal standard. The brains were first pretreated by homogenization in water and then extraction by 1 M HCl and then 3 M NaOH. The brain samples were extracted in diethyl ether and derivatized with hexafluoro-acetyl acetone (Laplanche & Morin, 1978. For detection of derivatives $^{m}/_{e}$ 418 was chosen for guanfacine and 424 for the internal standard. Under these conditions the calibration curves are linear, with thresholds of detection of about 0.4 ng/ml for plasma and 2.5 ng/g for brain. Each sample was determined twice.

Statistics

Statistical significances were calculated by unpaired Student's test, except for the salivation experiments in which the paired test was used.

Results

Effects of intravenous injections

Both drugs produced a biphasic effect on blood pressure, seen as an initial pressor effect followed by a reduction in MAP to below control values (Figure 1). Clonidine (30 μg/kg) produced a mean rise in pressure of 24 mmHg ($P<0.001$) by 30 s; however by 2 min mean arterial pressure had fallen to 9 mmHg below control values ($P<0.05$) and remained so for more than 60 min. The maximum fall was 13 mmHg at 10 min. After 90 min there was no significant difference from control values.

Guanfacine caused a rise, followed by a fall, in MAP, the magnitude and duration of both these effects being dose-dependent (Figure 2). After 30 μg/kg of guanfacine there was a mean rise in MAP of 14 mmHg which quickly returned to pre-injection levels. There was no significant hypotensive effect at this dose. Guanfacine 300 μg/kg produced a mean rise of 45 mmHg, the MAP remaining significantly greater than controls for 15 min followed by significant hypotension ($P<0.05$) 2 h after injection. The pressure remained below control values until 4 h after injection ($P<0.01$), rising towards control values thereafter. At a dose of 1 mg/kg guanfacine caused a mean rise in pressure of 44 mmHg, the MAP fell thereafter remaining significantly greater than controls for 60 min, becoming significantly lower than controls 3 h after injection and remaining so 7 h after injection.

Both clonidine (30 μg/kg) and guanfacine (300 μg/kg) caused rapid falls in heart rate (HR) of

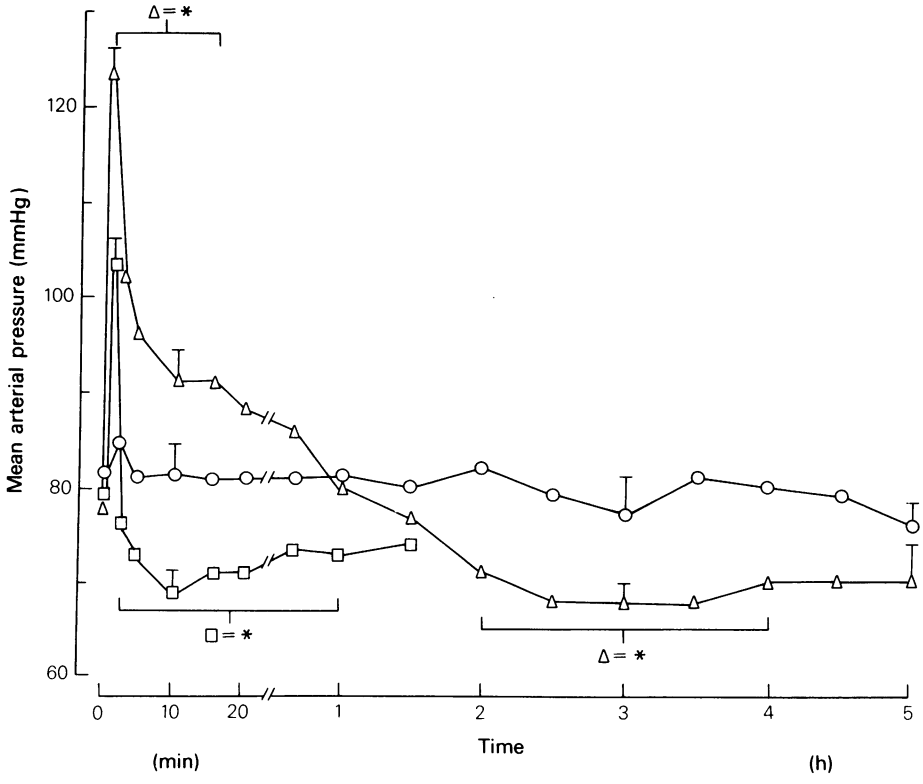

Figure 1 Effects of saline (○), clonidine (□) 30 μg/kg and guanfacine (△) 300 μg/kg, injected i.v., on mean arterial pressure in the conscious rabbit. Values shown are mean for 6 animals; vertical lines show s.e.mean. *Signifies $P < 0.05$ when compared to saline by Student's t test.

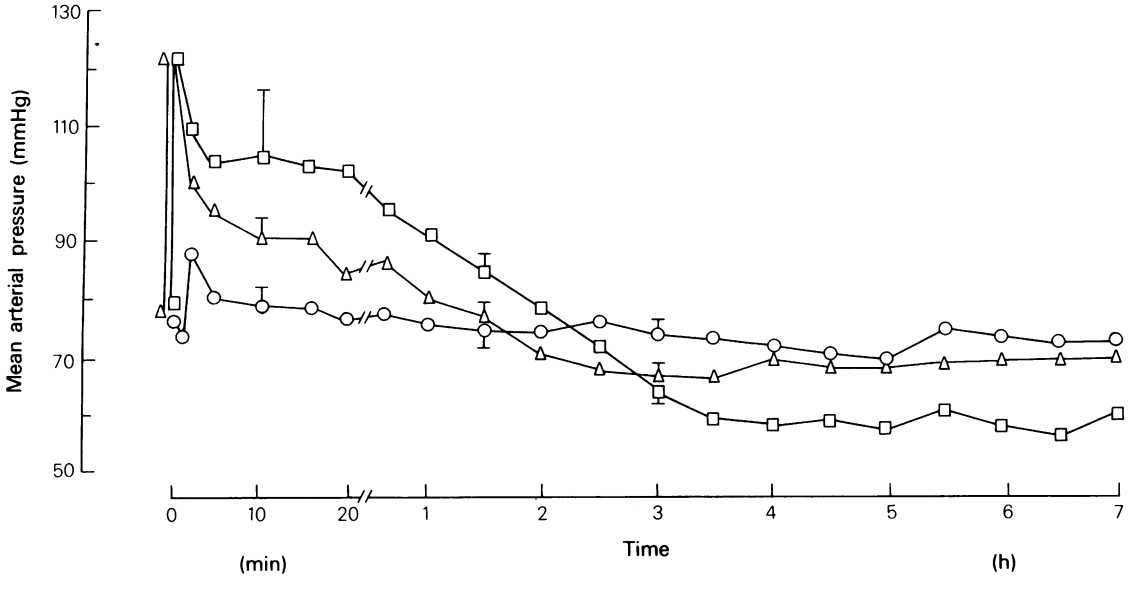

Figure 2 Effects of guanfacine in doses of 30 μg/kg (○), 300 μg/kg (△), and 1000 μg/kg (□) injected i.v. on mean arterial pressure in the conscious rabbit. Values shown are mean for 6 animals; vertical lines show s.e.mean.

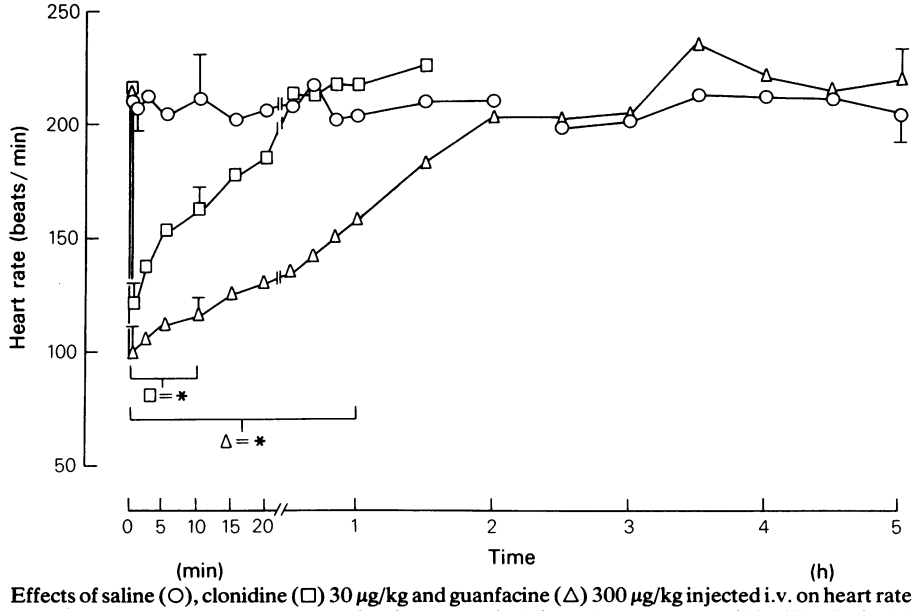

Figure 3 Effects of saline (○), clonidine (□) 30 μg/kg and guanfacine (△) 300 μg/kg injected i.v. on heart rate in the conscious rabbit. Values shown are mean for 6 animals; vertical lines show s.e.mean. *Signifies $P < 0.05$ when compared to saline by Student's t test.

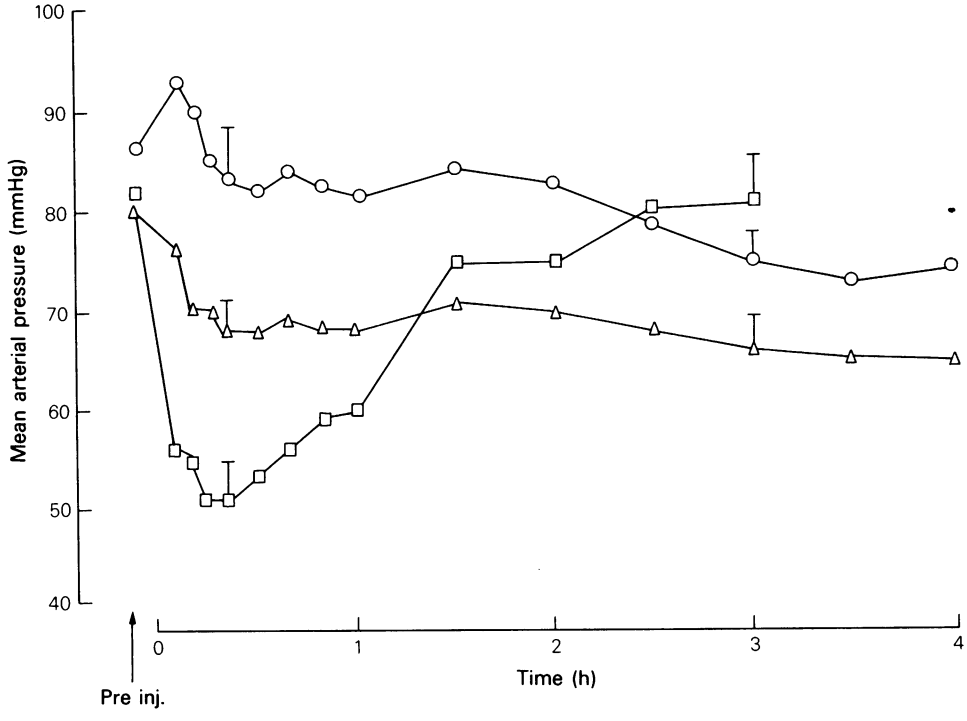

Figure 4 Effects of saline (○), clonidine (□) 3 μg/kg and guanfacine (△) 12 μg/kg, administered intracisternally on mean arterial pressure in the rabbit anaesthetized with pentobarbitone. Values shown are mean for 6 animals; vertical lines show s.e.mean.

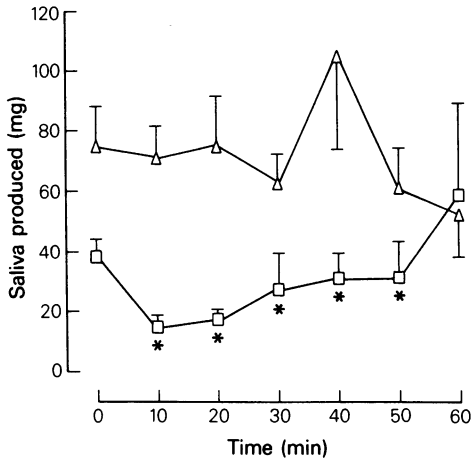

Figure 5 Effect of clonidine 30 μg/kg i.v. (□) or saline (△) on saliva production of the rabbit. Values shown are mean for 8 animals; vertical lines show s.e.mean. *$P<0.05$.

84 beats/min and 113 beats/min respectively ($P<0.01$), 30 s after injection (Figure 3). The HR rose thereafter until control levels were reached. The HR remained significantly depressed ($P<0.05$) 10 min after clonidine injection and 60 min after guanfacine injection.

Effects of intracisternal injections

Clonidine (3 μg/kg) lowered MAP compared to control animals, 2 min after injection (mean fall 29 mmHg, $P<0.05$), the maximum fall (34 mmHg, $P<0.01$) was attained 15 to 20 min after injection, and was maintained below controls at 60 min ($P<0.01$); however, it was not significantly lower at 90 min. Guanfacine (12 μg/kg) produced a gradual, smaller and more prolonged fall in MAP, being significantly lower after 5 min (mean fall 17 mmHg, $P<0.01$) and remaining so 150 min after injection ($P<0.05$) but not 180 min after (Figure 4).

Clonidine (3 μg/kg) caused a significant fall in HR 2 min after injection (mean fall 46 beats/min, $P<0.05$), the fall being maximal after 20 min (68 beats/min, $P<0.01$), remaining significantly lower at 90 min ($P<0.05$) but not 120 min. Guanfacine (12 μg/kg) produced little effect on HR for the first hour after injection, and was only significantly lower than saline-injected control animals at three time points: 90 ($P<0.05$), 150 ($P<0.05$) and 180 min ($P<0.01$).

Higher doses of guanfacine (15 μg/kg) caused death with respiratory depression 2–10 min after injection.

Salivation

Clonidine (30 μg/kg i.v.) significantly reduced salivation ($P<0.05$) for 50 min after injection, when compared with control (Figure 5). The response was strongest initially, gradually wearing off. The effect was dose-dependent, injection of 100 μg/kg i.v. still

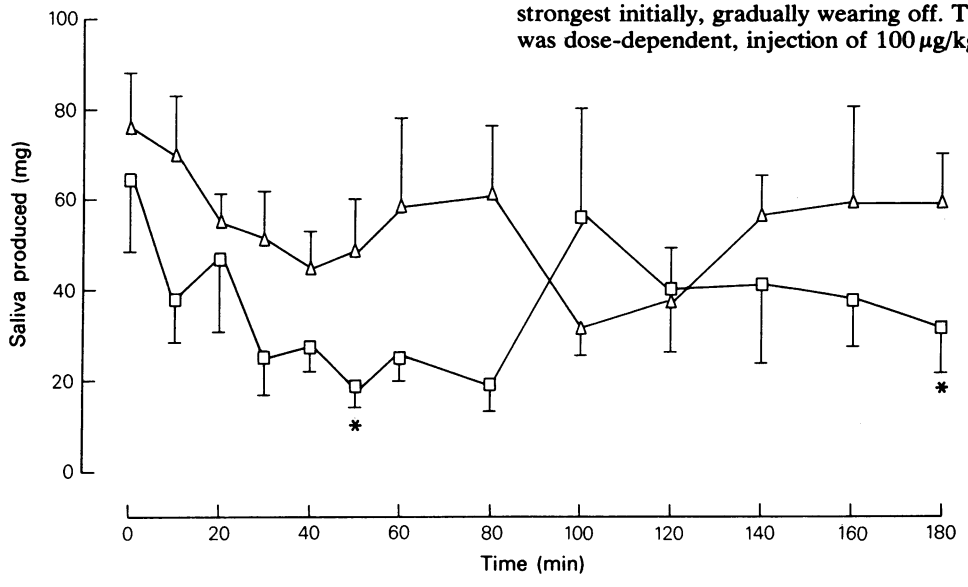

Figure 6 Effect of guanfacine 300 μg/kg i.v. (□) or saline (△) on saliva production in the rabbit. Values shown are mean for 8 animals; vertical lines show s.e.mean. *$P<0.05$.

produced significant xerostomia ($P<0.01$) 60 min after injection. An equihypotensive dose of guanfacine (300 μg/kg i.v.) only produced a significant fall ($P<0.05$) at two time points during the study, these being 50 and 180 min after injection (Figure 6).

Apparent partition coefficient

The mean of 3 estimations of the apparent partition coefficient, calculated for an octanol/buffer system at pH 7.4, was 5.4 for clonidine (range 5.1–5.9) and 21.2 for guanfacine (range 20.7–21.9). The figures given represent the amount of the drug found in the octanol divided by the amount in the phosphate buffer; hence guanfacine is more lipophilic in this system.

Blood:brain ratios

The levels of guanfacine found both in plasma and brain can be seen in Table 1. The plasma concentration fell rapidly with time over 3 h. The brain concentration stayed fairly constant, and the level after 90 min was significantly higher ($P<0.05$) than at either 15 or 180 min, suggesting that the concentration of guanfacine in the brain reaches a peak between 15 and 180 min after injection. The ratio of concentrations in brain and plasma increased throughout the period measured.

Discussion

Both the α_2-receptor agonists, clonidine and guanfacine, after intravenous injection, caused a biphasic effect on blood pressure over the dose-range given; however, the pressor and depressor effects due to guanfacine were of longer duration than those of clonidine. The effects of the drugs on salivation correlate with the clinical observations of Dollery & Davies (1980), in which clonidine caused a rapid, intense, short lasting xerostomia, whereas guanfacine does not cause it to the same extent, although its effects are of longer duration.

The rise in MAP is due to a peripheral pressor mechanism, as we have shown that a hypotensive effect can be obtained by an i.c. injection without any initial pressor effect. This pressor effect is due to stimulation of postsynaptic α-receptors in the vasculature, which may be of the α_1- or α_2-type (Timmermans & van Zwieten, 1981) and is presumably concurrent with the central hypotensive actions, assuming the drugs reach the brain rapidly. The overall effect on MAP is therefore the summation of these opposite peripheral and central effects. The central effects, which are a result of α_2-receptor stimulation, rapidly predominate. This effect probably has two causes: the first being that the level of drug in brain is higher than that in plasma (Cho & Curry, 1969) and the second being that as the plasma concentration rapidly falls there is less stimulation of α_1-receptors mediating vasoconstriction.

Both drugs reduced heart rate when equihypotensive doses were given i.v.; however, the effect due to guanfacine was of longer duration. The fall in heart rate is partly due to secondary baroreflex activity compensating for the initial pressor effect and, in the case of clonidine, partly to a direct central bradycardic effect of the drug. Although i.v. guanfacine caused a longer bradycardia than i.v. clonidine, this ceased at about the time the pressor effect ended. Intravenous clonidine produced a significant bradycardia of 10 min duration, but its pressor effect lasted less than 2 min, indicating a supplementary effect. The response to intracisternal injection of clonidine, which caused no pressor effect, supports this conclusion as it caused a significant bradycardia of over 90 min duration; i.c. guanfacine only showed cardiac slowing much later, at a point when the animal was emerging from the anaesthetic. As yet we have no explanation for this difference between the drugs.

The longer duration of the effects of guanfacine on MAP do not appear to be due to its slow access to the brain. Fifteen minutes after i.v. injection, the level of guanfacine in the brain is about twice as high as that in plasma. Three hours after injection the brain con-

Table 1 Estimates of the concentration of guanfacine in plasma and brain after an intravenous injection of guanfacine hydrochloride 300 μg/kg

Time (min)	*Plasma* (ng/ml)	*Brain* (ng/g)	*Brain/plasma*
15	55.70 ± 19.26	110.00 ± 18.89	2.11 ± 0.52
90	26.82 ± 4.33	140.32 ± 20.08*	5.27 ± 0.55
180	8.50 ± 2.87	108.78 ± 12.29	13.58 ± 3.11

There were five animals at each time point and the values given are mean ± s.d. The brain to plasma ratio is the mean ± s.d. of the individual ratios. *Signifies that the concentration in brain is significantly higher ($P<0.05$) 90 min after injection when compared with values at 15 and 180 min.

centration is about the same, whereas the plasma concentration has fallen markedly. Were its brain levels merely a reflection of its lipophilicity, shown by its octanol buffer partition coefficient, then they would have fallen in parallel with the plasma levels. It therefore appears that differences in duration of effects on MAP have a pharmacokinetic basis.

The longer pressor effects of guanfacine may be due to its longer plasma half-life, this being about 130 min in the rabbit (Barber & Reid, unpublished) compared with clonidine which has a half life of about 45 min in the rabbit (Reid, Barber & Davies, 1980). This would result in the guanfacine concentration in plasma persisting at levels high enough to stimulate peripheral α-receptors and hence delay the onset of the hypotensive effect.

The longer duration of the hypotensive effect of guanfacine thus appears to be due to the brain acting as a separate compartment. Kiechel (1980) reports a plasma terminal half life of 17 h in man for guanfacine, which may reflect this. Its levels in the brain are approximately the same after 15 min when the animal is hypertensive, at 90 min when it is normotensive, and at 180 min when it is hypotensive; however, the pressor effect gradually fades as the plasma concentration falls. This long plateau in levels at its site of hypotensive action must contribute to its greater length of hypotensive action, this being of at least 2 h duration, about twice that of clonidine.

This longer duration of action is of relevance clinically, not only because the drug may be given less frequently, but also because any interruption of treatment will be accompanied by a more gradual reversal of drug effect over 2–3 days (Reid, Zamboulis & Hamilton, 1980), and this may lead to less frequent withdrawal reaction.

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References

BREAM, J.B., LAVENER, M., PICARD, C.W., SCHOLTYSIK, G. & WHITE, T.G. (1975). Substituted phenylacetyl guanidines; a new class of antihypertensive agents. *Arzneim. Forsch.*, **25**, 1477–82.

CHALMERS, J.P. & WURTMAN, R.J. (1971). The fate of intracisternally administered norepinephrine-^{3}H in the brain and spinal cord of the rabbit. *J. Pharmac. exp. Ther.*, **178**, 819.

CHALMERS, J.P. & REID, J.L. (1972). Participation of central noradrenergic neurones in arterial baroreceptor reflexes in the rabbit. A study with intracisternally administered 6-hydroxydopamine. *Circulation Res.*, **31**, 789–804.

CHO, A.K. & CURRY, S.H. (1969). The physiological disposition of 2-(2,6-dichloranilino(2-imidazoline (St-155). *Biochem. Pharmac.*, **18**, 511–520.

DOLLERY, C.T., DAVIES, D.S., DRAFFAN, G.H., DARGIE, H.J., BEAN, C.R., REID, J.L., CLARE, R.A. & MURRAY, S. (1976). Clinical pharmacology and pharmacokinetics of clonidine. *Clin. Pharmac. Therap.*, **19** (1): 11–17.

DOLLERY, C.T. & DAVIES, D.S. (1980). Centrally acting drugs in antihypertensive therapy. *Br. J. clin. Pharmacol.* (Suppl. 1) 5S–12S.

DUBACH, U.C., HUWYLER, R., RADIELOVIC, P. & SINGEISEN, M. (1977). A new centrally acting antihypertensive agent. Guanfacine (BS 100–141) *Arzneim. Forsch.*, **27** (i): 674–676.

JERIE, P. (1980). Clinical experience with guanfacine in long-term treatment of hypertension. Part II: adverse reactions to guanfacine. *Br. J. clin. Pharmac.*, **10** (Suppl. 1) 1575–1645.

KIECHEL, J.R. (1980). Pharmacokinetics and metabolism of guanfacine in man: a review. *Br. J. clin. Pharmac.*, **10** (Suppl. 1), 253–325.

LAPLANCHE, R. & MORIN, Y. (1978). Automated determination of Estulic in biological fluids using a selected ion monitoring technique and an operator controlled data processing. *Quantitative Mass-Spectrometry in Life Sciences*, **11**, 339–345.

REID, J.L., BARBER, N.D. & DAVIES, D.S. (1980). The clinical pharmacology of clonidine: relationship between plasma concentration and pharmacological effect in animals and man. *Archs. Int. Pharmacodyn., Ther.* (Suppl.), 11–16.

REID, J.L., ZAMBOULIS, C. & HAMILTON, C.A. (1980). Guanfacine: effects of long-term treatment and withdrawal. *Br. J. clin. Pharmac.*, **10** (Suppl. 1) 183S–188S.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981). The postsynaptic α_2-adrenoceptor. *J. Auton. Pharmac.*, **1**, 171–183.

SCHOLTYSIK, G., LAUENER, H., EICHENBERGER, E., BURKI, H., SALTMANN, R., MULLER-SCWEINITZER, E. & WAITE, R. (1975). Pharmacological actions of the antihypertensive agent N-amidino-2-(2,6-dichlorophenyl) acetamide hydrochloride (BS 100–141). *Arzneim. Forsch.*, **25**, 1483–1491.

ZAMBOULIS, C., REID, J.L. & HAMILTON, C.A. (1978). BS 100–141: a centrally acting antihypertensive drug: effects on blood pressure and plasma and urinary catcholamines in essential hypertension. *Seventh Int. Congr. Pharmac. (IUPHAR)* Paris (1978).

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