Discussion

The maximum effect of Azone on the permeability of 5-FU across the hairless rat skin was to increase it about 100-fold. With multiple drug applications, however, low fluxes during the lag time may be ignored after the second administration. The small effect of Azone on the permeability across the stripped skin suggests that Azone mainly affects the stratum corneum. Azone seems to change the diffusivity of 5-FU in the stratum corneum and is not so effective against the diffusivities in the epidermis and dermis. Therefore, Azone would be useful for enhancing the permeability of hydrophilic compounds such as 5-FU, because the rate limiting layer for the percutaneous absorption of such drugs is the stratum corneum. In contrast, Azone might not be effective for enhancing the permeability of lipophilic compounds due to their intrinsic large permeability of the stratum corneum (Flynn et al 1981).

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Location of the mechanism of the clonidine withdrawal tachycardia in rats

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Withdrawal of chronic infusion of clonidine elicits severe tachycardia and short-lasting blood pressure elevations (upswings). Withdrawal of clonidine in low dosage (30 μg kg^{-1} day^{-1} i.c.v., 7 days) elicited a maximum of 10·9 ± 0·5 upswings h^{-1}. Cessation of s.c. infusion of clonidine (30 μg kg^{-1} day^{-1} 7 days) evoked a maximum of 1·9 ± 0·5 upswings h^{-1}. After cessation of the two clonidine infusions no overshoot of heart rate occurred. Withdrawal of a higher dose of clonidine (300 μg kg^{-1} day^{-1} s.c., 7 days), however, induced tachycardia (from 302 ± 8 to 433 ± 8 beats min^{-1}) and 7·6 ± 1·4 upswings h^{-1}. The administration of the α_2-adrenoceptor antagonist yohimbine precipitated withdrawal tachycardia in animals treated with oxymetazoline, a hydrophilic α-adrenoceptor agonist. Yohimbine (3 mg kg^{-1} i.p.) precipitated a severe rise in heart rate from 285 ± 14 to 520 ± 5 beats min^{-1} in oxymetazoline (300 μg kg^{-1} day^{-1} s.c., 7 days) treated rats and from 320 ± 13 to 420 ± 11 beats min^{-1} in saline-treated animals. Upswings were not induced by yohimbine treatment. It is concluded, that the blood pressure upswings after clonidine withdrawal are due to a central mechanism, whereas the mechanism of the overshoot of heart rate is located peripherally, probably at the cardiac presynaptic level.

Discontinuation of chronic treatment with clonidine (500 μg kg^{-1} day^{-1} s.c., 12 days) in normotensive rats elicits severe tachycardia (from 313 ± 4 to 456 ± 5 beats min^{-1}) and short-lasting intermittent blood pressure elevations (upswings) (8·5 ± 0·9 h^{-1}) (Thoolen et al 1981). The blood pressure upswings appear to be of central origin, since i.c.v. injection of the hydrophilic imidazolidine derivative oxymetazoline (30 μg kg^{-1}) abolishes the upswings after clonidine withdrawal (500 μg kg^{-1} day^{-1} s.c., 12 days), whereas i.p. administered oxymetazoline (30 μg kg^{-1}) does not affect the frequency of the upswings (Thoolen et al 1983). An additional argument in favour of a central mechanism is the suppression of the upswings by i.c.v. injection of 0·3–10 μg kg^{-1} morphine, whereas s.c. administered morphine supresses the upswings at 1–3 mg kg^{-1} (Thoolen et al 1983).

The origin of the withdrawal tachycardia is less clear. Morphine (0·3–10 μg kg^{-1} i.c.v. or 1–3 mg kg^{-1} s.c.) does not suppress the withdrawal tachycardia, but oxymetazoline (30 μg kg^{-1} day^{-1} i.c.v. or i.p.) abolishes this effect (Thoolen et al 1983). This finding points towards a possible involvement of a peripherally located mechanism in the development of the withdrawal tachycardia.

To elucidate the locations of the withdrawal mechanisms, the effects on blood pressure and heart rate of discontinuation of i.c.v. and s.c. infusions of clonidine were studied. In contrast to clonidine, the α-adrenoceptor agonist oxymetazoline does not cause withdrawal symptoms (as such) upon cessation of treatment, due to its long half life. For this reason withdrawal phenomena were provoked by means of α_2-adrenoceptor blockade with yohimbine.

Materials and methods

Male, normotensive Wistar rats (350–400 g, Cpb/Wv-strain, TNO, Zeist, The Netherlands) were used. Permanently indwelling catheters were implanted under hexobarbitone sodium anaesthesia (150 mg kg^{-1} i.p.) in

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the abdominal aorta, according to the method of Still & Whitcomb (1956), and modified by Weeks & Jones (1960). Catheters were flushed daily with heparinized saline (100 IU ml^{-1}). Blood pressure and heart rate were recorded via these catheters by means of Statham P23Db pressure transducers, and recorded on a Hellige HE19 polygraph. Alzet 2002 osmotic minipumps (delivery rate 0·5 μ h^{-1}) were used to infuse the drugs.

Subcutaneous administration. Two days after aortic catheter implantation, an Alzet osmotic minipump was implanted s.c. in the dorsal region under diethyl ether anaesthesia. The drugs were infused s.c. via the pumps in the following dosage: clonidine, 30 or 300 μg kg^{-1} day^{-1}, or oxymetazoline, 300 μg kg^{-1} day^{-1}. Blood pressure and heart rate were measured daily between 14.00 and 17.00 h. After 7 days the clonidine infusion was discontinued abruptly by removing the clonidine pumps. The clonidine withdrawal was followed by a continuous registration of blood pressure and heart rate during the following 13 h and later, as indicated. The effect of oxymetazoline was terminated by injection of yohimbine (3 mg kg^{-1} i.p.), followed by continuous recording of blood pressure and heart rate during 90 min. The same procedure was used for animals after 7 days of 0·9% NaCl (saline) infusion.

Intracerebroventricular administration. Two days after the aortic catheter implantation, anaesthesia was induced with hexobarbitone sodium (150 mg kg^{-1} i.p.) and the skull exposed in the region of the bregma via a medial skin incision. The periosteum was removed and a stainless steel tube (o.d. 0·9 mm, i.d. 0·6 mm) was introduced via a 1 mm hole, made by means of a dental drill, 1·5 mm lateral and 0·5 mm rostral from the bregma, until the tip was 3·5 mm below the surface of the dura. The stainless steel tube was anchored with a stainless steel screw and dental acrylic. A PE20 catheter (o.d. 1·09 mm, i.d. 0·38 mm) was connected to the stainless steel tube. The other end of the catheter was connected to an Alzet 2002 osmotic minipump which was situated subcutaneously in the dorsal region. This pump infused via the PE20 catheter clonidine (30 μg kg^{-1} day^{-1}) into the right lateral ventricle. Blood pressure and heart rate were measured daily between 1400 and 1700 h. After 7 days of infusion, the minipumps were removed from the conscious animals and the PE20 catheter was disconnected from the minipump. The lumen of the remaining part of the catheter was closed by melting the catheter tip with a soldering iron. After the discontinuation of the infusion, blood pressure and heart rate were measured continuously during the following 13 h and later, as indicated.

Drugs used were: clonidine HCl and oxymetazoline (gifts: C. H. Boehringer Sohn, Ingelheim, FRG), yohimbine HCl (Sigma, St Louis, USA), hexobarbitone sodium (OPG, Utrecht, The Netherlands), heparin (Novo Industri A/S, Copenhagen, Denmark).

Data presented are means ± s.e.m. Statistical significance was evaluated by means of analysis of variance and a *t*-test (Wallenstein et al 1980).

Results

During a 7 day infusion of oxymetazoline (300 μg kg^{-1} day^{-1} s.c.) or clonidine (30 or 300 μg kg^{-1} day^{-1} s.c. or 30 μg kg^{-1} day^{-1} i.c.v.) blood pressure and heart rate remained unchanged.

Withdrawal of a 7 day s.c. infusion of clonidine (300 μg kg^{-1} day^{-1}) induced a tachycardia (from 302 ± 8 to 433 ± 8 beats min^{-1}, Fig. 1) and evoked 7·6 ± 1·4 upswings h^{-1}.

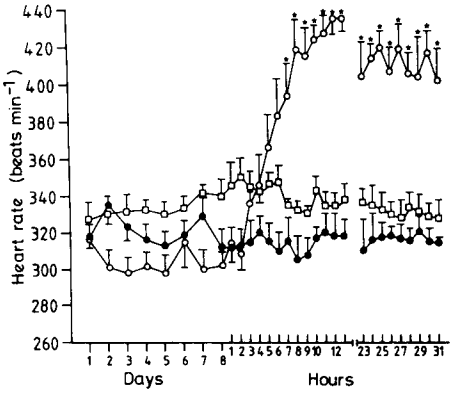

FIG. 1. Effect on heart rate of continuous infusion and withdrawal of clonidine (30 μg kg^{-1} day^{-1} s.c. (●), 30 μg kg^{-1} day^{-1} i.c.v. (□) and 300 μg kg^{-1} day^{-1}, s.c. (○) in conscious normotensive rats. Symbols represent mean values ± s.e.m. (n = 8–10). $^*P < 0.05$.

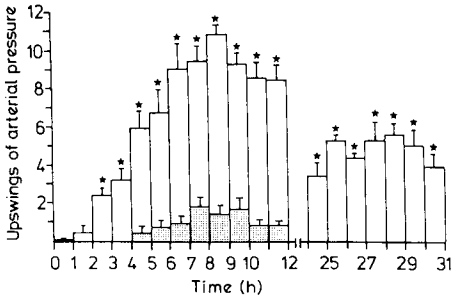

FIG. 2. Incidence of blood pressure upswings after withdrawal of clonidine infusion (30 μg kg^{-1} day^{-1} i.c.v., open columns and 30 μg kg^{-1} day^{-1} s.c., dotted columns) in conscious normotensive rats. Symbols represent mean values ± s.e.m. (n = 8–10). $^*P < 0.05$.

Cessation of s.c. infusion of clonidine (30 μg kg^{-1} day^{-1}, 7 days) caused 1·9 ± 0·5 upswings h^{-1} maximally (Fig. 2). Discontinuation of i.c.v. infusion of clonidine (30 μg kg^{-1} day^{-1}, 7 days) elicited 10·9 ± 0·5 upswings h^{-1} (Fig. 2). In both groups no withdrawal tachycardia occurred (Fig. 1).

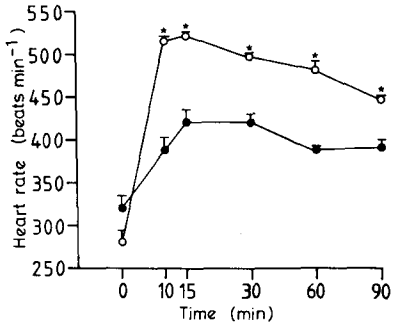

FIG. 3. Effect on heart rate of yohimbine (3 mg kg^{-1} i.p.) in conscious normotensive rats, after 7 days of infusion with oxymetazoline (300 μg kg^{-1} day^{-1} s.c. (○)) or saline (●). Symbols represent mean values ± s.e.m. (n = 6–8). *$P <$ 0·05.

Injection of yohimbine (3 mg kg^{-1} i.p.) in rats, on the 7th day of oxymetazoline infusion evoked a severe tachycardia (from 285 ± 14 to 520 ± 5 beats min^{-1}), whereas in animals on the 7th day of saline infusion only a reflex tachycardia (from 320 ± 13 to 420 ± 11 beats min^{-1}), due to the vasodilator effect of yohimbine, was observed (Fig. 3). Yohimbine did not induce upswings in oxymetazoline-treated rats.

Discussion

Discontinuation of the i.c.v. infusion of the low dose of clonidine (30 μg kg^{-1} day^{-1}) or s.c. infusion of the high dose of clonidine (300 μg kg^{-1} day^{-1}) evoked a high frequency of blood pressure upswings, whereas withdrawal of s.c. infusion of low dose of clonidine hardly caused upswings. These results are in accordance with the observation that central infusion of low doses of morphine or peripheral administration of 300 times higher doses of morphine suppresses the upswings after clonidine withdrawal (Thoolen et al 1983). Both series of experiments suggest that the upswings are caused by a central mechanism. But, it could not be decided whether the clonidine withdrawal tachycardia in rats is caused by a centrally or peripherally located mechanism. As shown in the present study, an overshoot of heart rate can be elicited by the interruption of the stimulation of peripheral α_2-adrenoceptors by the hydrophilic imidazolidine derivative oxymetazoline. This interruption was precipitated by yohimbine. Similar tachycardic effects were observed upon withdrawal of high dose of clonidine. Cessation of i.c.v. or s.c. infusion of low dose of clonidine did not induce withdrawal tachycardia. The absence of upswings after interruption of oxymetazoline treatment is readily explained by the poor penetration of oxymetazoline into the brain, since we assume that the upswing phenomenon is initiated at the level of the CNS. Furthermore, these findings indicate that peripherally localized α_2-adrenoceptors play a part in the development of the overshoot of heart rate. De Jonge et al (1981) showed that stimulation of cardiac presynaptic α_2-adrenoceptors significantly contributes to the acute bradycardic effect of clonidine in the spontaneously hypertensive rat.

We conclude that the blood pressure upswings are due to a centrally located mechanism, whereas the withdrawal tachycardia is more likely to be initiated at peripheral sites, probably at a presynaptic level in the heart.

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