FIG. 2. The record of a preliminary experiment illustrating the effect of indomethacin at 10 mg kg^{-1} i.v. on the pulsatile blood pressure, heart rate, and mean blood pressure responses of an anaesthetized dog to intravenous substance P (SP) at 3 $\times$ 10^{-10} mol kg^{-1} (404 ng kg^{-1}) and arachidonic acid at 1 mg kg^{-1} (aa 1) and 2 mg kg^{-1} (aa 2). The time marks indicate 1 min intervals.

depressor responses, over the dose range studied, are not due partially or wholly to cholinergic, histaminergic, or β-adrenergic receptor stimulation. This confirms the conclusions reached by Pernow & Rosell (1975) from experiments on the mechanism of the effect of substance P on blood flow in canine adipose tissue and skeletal muscle. Eklund et al (1977) had observed that indo-

methacin did not alter the increase in human forearm blood flow during intravenous infusion of substance P. It was concluded that the vasodilation elicited by substance P was not mediated by the release of prostaglandins but rather was due to a direct effect of substance P on vascular smooth muscle. That this conclusion may not be relevant under all experimental conditions and in all species is evidenced by the results of the present study which clearly indicates that prostaglandins do participate in the vasodepressor responses to some doses of substance P in the anaesthetized dog.

REFERENCES

Burcher, E., Atterhög, J. H., Pernow, B., Rossell, S. (1977) in: von Euler, U. S., Pernow, B. (eds) Substance P, Raven Press, New York, 261–268

Bury, R. W., Mashford, M. L. (1977) Eur. J. Pharmacol. 45: 335–340

Eklund, B., Jogestrand, T., Pernow, B. (1977) in: von Euler, U. S., Pernow, B. (eds) Substance P, Raven Press, New York, 275–285

Flower, R. J. (1974) Pharmacol. Rev. 26: 33–67

Hallberg, D., Pernow, B. (1975) Acta Physiol. Scand. 93: 277–285

Losay, J., Mroz, E. A., Tregear, G. W., Leeman, S. E., Gamble, W. J. (1977) in: von Euler, U. S., Pernow, B. (eds) Substance P, Raven Press, New York, 287–293

Pernow, B., Rosell, S. (1975) Acta Physiol. Scand. 93: 139–141

Traczyk, W. Z. (1977) in: von Euler, U. S., Pernow, B. (eds) Substance P, Raven Press, New York, 297–309

Potentiation of apomorphine-induced rotational behaviour by naloxone

RAYMOND M. QUOCK*, TERRENCE B. WELSH, *Division of Pharmacology, Department of Basic Sciences, Marquette University School of Dentistry, Milwaukee, Wisconsin 53233, U.S.A.*

Previous research in our laboratories has established that pretreatment of animals with narcotic antagonist drugs produces significant enhancement of the effects of dopamine mimicking drugs. The hyperthermic effect of the dopamine mimicking drug apomorphine is increased by 50% if rabbits are pretreated systemically or centrally with naloxone (Quock 1977). Naloxone and naltrexone both significantly potentiate the anticataleptic activity of L-dopa in reserpinized mice (Namba et al 1980). This present communication reports our findings in still another experimental paradigm of central dopamine activity, apomorphine-induced rotational behaviour in rats with unilateral lesions of the nigrostriatal pathway.

Twelve male Sprague-Dawley rats (200–300 g, Gibco Animal Resources, Madison, Wisconsin) were anaesthetized with pentobarbitone and mounted in a stereotaxic headholder (David Kopf Instruments,

Tujunga, California). A Radionics RFG-4 radiofrequency lesion generator was used to produce electrocoagulation lesions at stereotaxic coordinates localizing the substantia nigra (A 2·9, V − 2·5, L $\pm$ 1·7) (Setler et al 1978). Beginning four days after surgery, animals were subjected to control apomorphine or naloxone/apomorphine experiments at four or five day intervals. The control experiments involved intraperitoneal injection of apomorphine 1·0 mg kg^{-1}, the animal was then placed into a rotometer for 30 min, during which rotations were recorded. The naloxone/apomorphine experiments involved intraperitoneal naloxone pretreatment at 1·0 mg kg^{-1} 5 min before the apomorphine challenge, followed by 30 min in the rotometer. Rats were tested with apomorphine or naloxone/apomorphine on an alternate basis for at least six total experiments (average: 7·8 $\pm$ 0·8 experiments per rat). Then they were killed for histological verification of the lesion sites.

Apomorphine hydrochloride (Merck) and naloxone

* Correspondence.

hydrochloride (Endo) were prepared in aqueous solution immediately before the experiments. The doses represent the weights of the salts. The volume of injection was 0·1 ml per 100 g.

The rotational indices of animals per 2 min intervals over 30 min were averaged and statistically evaluated, using a paired t-test (Goldstein 1964).

Rats treated with intraperitoneal injections of 1·0 mg kg^{-1} of naloxone alone failed to exhibit rotational behaviour for up to 30 min following injection. Rats treated with apomorphine alone displayed significant rotational behaviour in an ipsilateral direction. Earlier experiments in which rats were challenged with apomorphine at four or five day intervals indicated no change in the rotational response to apomorphine itself over successive trials. Naloxone pretreatment did not alter the direction of turning but did produce a significant enhancement of the intensity of apomorphine-induced rotational behaviour (Table 1).

Unilateral lesions of the dopamine nigrostriatal system are reported to result in denervation supersensitivity of the striatal receptors on the side of the lesion (Ungerstedt 1971). Challenging such animals with a direct acting dopaminomimetic drug like apomorphine should evoke strong contralateral rotational behaviour. The present investigation reports that rats turned in an ipsilateral direction, a paradoxical finding that is nonetheless consistent with other reports (Costall et al 1975; Costall & Naylor 1975).

It has been speculated that the relative non-specificity of electrocoagulation lesions (as opposed to the more

Table 1. The influence of naloxone on apomorphine-induced rotational behaviour in rats with unilateral lesions of the nigrostriatal system. All rats received 1·0 mg kg^{-1} of apomorphine following either no pretreatment or 1·0 mg kg^{-1} of naloxone. The data are mean number of rotations $\pm$ s.e.m.

Time interval (min)	After apomorphine alone	After naloxone and apomorphine
0–2	0·23 ± 0·14	0·25 ± 0·17
2–4	4·14 ± 1·06	5·14 ± 1·29
4–6	7·79 ± 1·33	10·82 ± 1·55**
6–8	9·51 ± 1·37	12·81 ± 1·67*
8–10	9·75 ± 1·15	13·30 ± 1·63**
10–12	9·96 ± 1·25	13·75 ± 1·49**
12–14	9·16 ± 1·26	12·83 ± 1·41*
14–16	8·99 ± 1·22	12·71 ± 1·27*
16–18	8·23 ± 1·02	12·31 ± 1·19**
18–20	7·87 ± 1·10	11·08 ± 1·06*
20–22	6·96 ± 1·07	10·17 ± 1·06**
22–24	6·48 ± 1·11	9·26 ± 1·13*
24–26	5·71 ± 1·06	8·72 ± 1·01*
26–28	5·36 ± 0·95	7·68 ± 1·06*
28–30	5·24 ± 0·92	7·24 ± 0·97*

A total of twelve rats were used, each being subjected to alternating apomorphine and naloxone/apomorphine experiments. Statistical significance was determined, using the paired t-test: *, $P < 0.01$; **, $P < 0.001$.

commonly employed 6-hydroxydopamine-induced chemical lesions) damage nerve tracts other than the dopamine nigrostriatal pathway which might also be essential to the manifestation of rotational behaviour (Glick et al 1976).

Despite the induction of ipsilateral rather than contralateral turning by apomorphine, our experiments do demonstrate that the pretreatment of lesioned rats with naloxone does produce a significant enhancement of the intensity of apomorphine-induced rotational behaviour. This is consistent with other observations in our laboratories that show narcotic antagonist potentiation of apomorphine-induced hyperthermia in rabbits (Quock 1977) and L-dopa reversal of reserpine-induced catalepsy in mice (Namba et al 1980). It has also been reported that naloxone enhances apomorphine- and piribedil-induced operant responding in rats (Harris et al 1977). On the other hand, these findings are not in agreement with reports from other laboratories. It has been found that naloxone antagonizes—rather than potentiates—apomorphine-induced stereotypic gnawing behaviour in guinea-pigs and rats (Margolin & Moon 1979; Moon et al 1980). The discrepancy between these various observations is currently unexplained, though it may be possible that the interaction between naloxone and drugs that mimic dopamine is manifested differently in various test systems.

One possible explanation for our observation of naloxone potentiation of apomorphine drug effect may involve naloxone blockade of opiate receptors that play regulatory roles upon dopamine neuronal function (Loh et al 1976). Interaction between naloxone and GABAergic mechanisms, which might also play a modulatory role upon dopamine neurons, is also possible (Dingledine et al 1978).

REFERENCES

Costall, B., Marsden, C. D., Naylor, R. J., Pycock, C. J. (1975) Br. J. Pharmacol. 55: 289P–290P

Costall, B. Naylor, R. J. (1975) Psychopharmacologia 41: 57–64

Dingledine, R., Iversen, L. L., Brueker, E. (1978) Eur. J. Pharmacol. 47: 19–27

Glick, S. D., Jerussi, T. P., Fleisher, L. N. (1976) Life Sci. 18: 889–896

Goldstein, A. (1964) Biostatistics: An Introductory Text. Macmillan New York

Harris, R. A., Snell, D., Loh, H. H., Way, E. L. (1977) Eur. J. Pharmacol. 43: 243–246

Loh, H. H., Brase, D. A., Sampth-Khaha, S., Mir, J. B., Way, E. L., Li, C. H. (1976) Nature 264: 567–568

Margolin, D. I., Moon, B. Y. (1979) J. Neurol. Sci. 43: 13–17

Moon, B. Y., Feigenbaum, J. J., Carson, P. E., Klawans, H. L. (1980) Eur. J. Pharmacol. 61: 71–78

Namba, M. M., Quock, R. M., Malone, M. H. (1980) Proc. West. Pharmacol. Soc. 23: 285–289

Quock, R. M. (1977) Life Sci. 20: 2005–2012

Setler, P. E., Malesky, M., McDevitt, J., Turner, J. (1978) Life Sci. 23: 1277–1284

Ungerstedt, U. (1971) Acta physiol. Scand. 82, Suppl. 367: 69–93

Affinity of butriptyline and other tricyclic antidepressants for α-adrenoceptor binding sites in rat brain

T. A. Pugsley*, W. Lippmann, *Biochemical Pharmacology Department Ayerst Research Laboratories, Box 6115, Montreal, Quebec, Canada, H3C 3J1*

Butriptyline is a tricyclic compound possessing a neuro-psychopharmacological profile in animals similar to that of various tricyclic antidepressants (Voith & Herr 1969; Herr et al 1971) and is a clinically effective anti-depressant agent (Ambrus 1971; Levinson 1974; Kapadia & Smith 1976; Brodie et al 1978; Burrows et al 1979). The drug does not appreciably block nor-adrenaline (NA) uptake in mouse and rat heart in vivo (Lippmann 1969, 1971) or NA and 5-hydroxytrypt-amine (5-HT) uptake in rat brain in vivo (Pugsley & Lippman 1974). In vitro, butriptyline inhibits [³H]-dopamine uptake in rat corpus striatum, the drug being similar in activity to maprotiline, trimipramine, iprindole, mianserine and the classical tricyclics and about 50 times less potent than nomifensine (Randrup & Braestrup 1977). Butriptyline and the other above-mentioned drugs exhibit only weak ability to inhibit in vitro [³H]5-HT uptake in rat whole forebrain and [³H]NA uptake in rat occipital cortex preparations in comparison with the classical antidepressants, e.g. chlorimipramine and desipramine, respectively (Randrup & Braestrup 1977).

U'Prichard et al (1978) have shown that the affinities of the classical tricyclic antidepressants for rat brain α-adrenoceptors, as judged by their ability to displace the postsynaptic α-adrenoceptor antagonist [³H]-WB-4101 2-(N-[2',6'-dimethoxyphenoxyethyl]) amino-methyl-1,4-benzodioxane [phenoxy-3-³H (N)], correlate directly with the ability of these agents to relieve psycho-motor agitation and to induce sedation and hypotension and inversely with their tendencies to elicit psychomotor activation. We have therefore determined the ability of butriptyline to interact with rat brain α-adrenoceptors labelled by [³H]WB-4101 and to compare the affinity of butriptyline with that of the classical antidepressants amitriptyline, imipramine and desipramine.

The α-adrenoceptor binding activity of the test drug was determined essentially as described previously (Greenberg et al 1976). Two rats were decapitated and the brains quickly removed. The cerebellum and brain stem were excised and the remainder of the brain homo-genized in 20 volumes of ice-cold 50 mM Tris-HCl

(pH 7·7; 25 C) with a Brinkman Polytron PT-10 for 10 s at setting No 6 and then centrifuged at 50 000 g for 10 min. The homogenate was washed once by resus-pension and centrifugation; the final suspension was in 50 volumes of cold 50 mM Tris-HCl buffer (pH 7·7; 25 C). In the standard binding assay, incubation tubes each received 1·0 ml of tissue suspension (20 mg, wet weight of original tissue), 100 μl (4·4 nM) of [³H]WB-4101 to give a final concentration of 0·22 nM, 100 μl of various concentrations of test drug prepared freshly in 0·1% ascorbic acid, 0·7 ml of Tris-HCl buffer (pH 7·7; 25 C) and 100 μl ascorbic acid. The extent of non-specific binding of [³H]WB-4101 was determined from parallel assay tubes which contained a large excess of ( )-NA (2 mM) in 100 μl of 0·1% ascorbic acid. Assays were conducted in triplicate; tubes were incubated for 15 min at 25 C with constant shaking. After the incubation, each sample was rapidly filtered, under reduced pressure, through a Whatman GF/B glass fibre filter. The incubation tube was washed twice with 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7·7; 25 C) and each wash filtered. Each filter was placed in a vial containing 10 ml of Aquasol and after shaking for 1 h, the radioactivity content was measured in a liquid scintillation spectro-meter. Specific binding for [³H]WB-4101 is defined as the total binding minus the binding obtained in the presence of 100 μM (−)-NA.

The IC50 (concentration producing 50% inhibition of the specific binding of [³H]WB-4101) for the test drug and the Hill coefficient were calculated by linear regression of the line obtained by plotting the log of [% B_{max}/100%−%B_{max}] versus the log of the concen-tration of inhibitor. B_{max} is taken as the specific binding occurring in the absence of displacing drug. Binding occurring in the presence of displacing drug is expressed as % B_{max}. The IC50 value is the point at which log of [% B_{max}/100%−%B_{max}] is 0 and the Hill coefficient is the slope of the line.

Butriptyline hydrochloride (Evadyne) was from Ayerst Laboratories. Desipramine hydrochloride (Pertofrane; Ciba-Geigy Ltd), imipramine hydro-chloride (Tofranil; Ciba-Geigy Ltd) and amitriptyline hydrochloride (Elavil; Merck, Sharpe & Dohme, Ltd) were gifts from the respective companies. [³H]WB-4101

* Correspondence.